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SOIL SCIENCE

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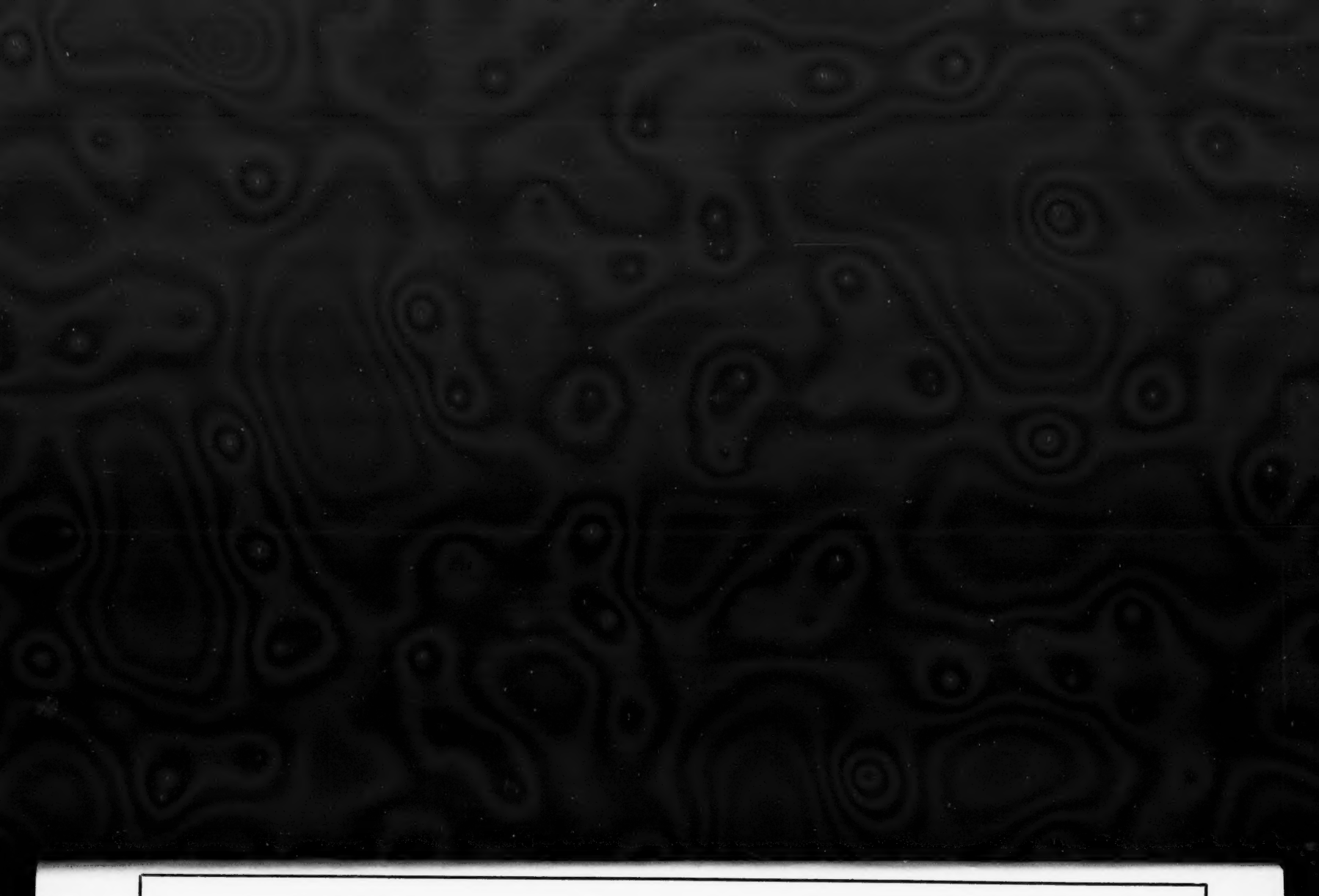

PRESERVATION OF SOILS AGAINST DEGENERATION[1]

W. L. POWERS

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Received for publication July 31, 1933

In previous reports (4, 5, 6) the writer has shown the importance of an intimate natural or artificial mixture of highly organic and nearly neutral colloidal matter in imparting high base exchange capacity, nitrogen content, and nutrient supplying power to soils.

The present study was undertaken to determine how much additions of lime, organic matter, and other soil building treatments used on some of the older experiment fields in the eastern states and in Oregon have affected the supply of total nitrogen, organic matter, and base exchange capacity of the soils of different regions. The old plat soils collected and analyzed included representatives from humid, semi-arid, and arid sections.

The work of Gedroiz (2) and that of Byers and Anderson (1) have shown striking differences in the clay fraction of the great soil groups such as are brought about by the process of podzolization under humid conditions with extensive leaching of the products of soil hydrolysis. The intensity of this process is recognized to be dependent upon the climatic conditions and related vegetation. Under tropical humid or perhaps alkaline conditions soil colloids tend to become largely decalcified or desilicated and the base adsorbing complex degenerated into simple oxides or end products with marked reduction in base exchange capacity. There is abundant evidence of a tendency to decrease soil nitrogen and organic matter by continuous cropping and cultivation in humid climates due to both increased bacterial action and alteration of the colloid complex. For example, Shutt (10) found Manitoba prairie soil nitrogen content to decrease from 0.651 to 0.506 per cent and loss on ignition to decrease from 19.43 to 14.79 per cent with 25 years of cultivation. The writer has previously noted (7) that organic matter and nitrogen can be more readily built up in calcareous soils under irrigation farming. Soil change due to long-continued cultivation has been the subject of a special committee of the American Soil Survey Association for some years and the present study was undertaken as a contribution of the writer to the work of that committee.

EXPERIMENTAL WORK

To determine the extent to which base exchange capacity and other soil characteristics may be affected by long-continued cultural treatments, a

[1] Published as technical paper 201 with the approval of the director as a contribution from the soils department, Oregon Agricultural Experiment Station.

chemical study was undertaken of soil samples from the older experiment stations.[2] The samples were prepared and analyzed by the current methods in use in the laboratories of the Oregon station perviously described (4).

The results of determinations with soils from Missouri, Ohio, and Illinois Station plats. Table 1 is arranged to show the value of additions of organic matter and lime in building up soils in long-time experiments. It will be seen from the first section of the table that the Wooster silt loam from the Wooster station has been improved in nitrogen and organic matter content by the addition of lime and floats, or by manuring, particularly in the surface soil. This has been accompanied by an increase in phosphate supplying power and has resulted in a maximum base exchange content for the plats represented. A definite increase in base exchange content has resulted from manuring.

Samples from the Morrow plats at the Illinois experiment station gave results as reported in the second section of table 1. Some increase in organic matter was found, where the soil was treated with lime, manure, and phosphorus under continuous corn culture, and this increase extends to subsoil samples taken at from 19 to 30 inches. The increase with this treatment, using a 2-year rotation corn-oats, has been about 50 per cent in organic matter and is noticeable to the 30-inch level. There has been a moderate increase in total nitrogen from these treatments, and this tends to extend into the subsoil. Base exchange content appears to have been affected but moderately by these treatments. Phosphate supplying power of the soil has been greatly improved.

At the Sanborn experiment field of the Missouri station manuring and rotation treatments are included. These have given a marked increase in organic matter and total nitrogen. In some cases the manuring treatment has practically doubled the supply of these constituents as compared to untreated checks. There has been an apparent build-up in base exchange capacity from manuring and apparently from rotation treatment. Manure has increased the available phosphorus. No comparison with the virgin soil has been attempted.

Soil changes from rotation, manure, and supplemental irrigation at Corvallis, Oregon. Striking increases in nitrogen and organic matter have previously been reported from the first 15 years of manuring, rotation, and supplemental irrigation in the bean rotation of the irrigation experiment field at the Oregon Agricultural Experiment Station at Corvallis. The soil is Amity silty clay loam, under a sub-humid climate. Further determinations reported in table 2 show the effect of pursuing these treatments for a period of 20 years, upon nitrogen, organic matter, and base exchange capacity. There has been a definite trend toward increased nitrogen and organic matter both with and with-

[2] Soil samples were kindly supplied by Professors M. F. Miller, R. S. Smith, and Earl E. Barnes of the Missouri, Illinois, and Ohio State Agricultural Experiment Stations, respectively. The writer is grateful to Clarence Burnham and J. L. Wursten, graduate assistants, for assistance in making determinations herein reported. Acknowledgment is made to Dr. H. G. Byers for reading and giving helpful criticisms of this article.

TABLE 1

Value of organic matter and lime in building up soils from long-time experiment fields—Ohio, Illinois, and Missouri Stations

PLAT	DEPTH	TREATMENT	pH	ORGANIC MATTER	TOTAL NITROGEN	BASE EXCHANGE CONTENT	AVAILABLE PHOSPHORUS
	inches			*per cent*	*per cent*	*m.e.: 100 gm.*	*p.p.m.*
			Ohio Station				
†14	0–7	Check	7.65	1.30	0.0896	18.04	9
14	7–12			0.65	0.0560	7.86	
14	12–20			0.57	0.0560	7.66	
†15	0–7	Manure	7.48	1.30	0.0980	27.70	9
15	7–12			1.00	0.0588	9.06	
15	12–20			0.83	0.0560	9.50	
*7	0–7	Check	6.25	1.20	0.0980	29.49	7
7	7–12	Lime accidentally		0.70	0.0420	6.84	
7	12–20	applied in 1929		0.70	0.0504	8.78	
6	0–7	Lime and manure	7.50	1.70	0.1036	45.74	12
6	7–12			0.80	0.0532	10.08	
6	12–20			0.56	0.0392	8.24	
10	0–7	Check	6.72	1.28	0.0784	17.95	7
10	7–12			0.66	0.0392	8.03	
10	12–20			0.65	0.0364	8.37	
			Illinois Station—Morrow Plats				
3 N.E.	0–8	Check, corn con-	5.30	2.10	0.1260	31.69	11
3 N.E.	8–19	tinuous		1.60	0.1170	16.96	
3 N.E.	19–30			0.90	0.0700	18.19	
3 S.E.	0–8	Manure, lime, phos-	5.50	2.40	0.1960	35.40	50
3 S.E.	8–19	phorus, corn con-		1.80	0.1372	12.55	
3 S.E.	19–30	tinuous		1.10	0.0700	16.27	
4 S.E.	0–8	Manure, lime, phos-	6.00	3.10	0.1848	27.18	55
4 S.E.	8–19	phorus, corn-oats		2.10	0.1568	11.08	
4 S.E.	19–30			2.00	0.1288	10.54	
			Missouri Station—Sanborn Field				
17	A_1	Corn continuous	5.35	0.97	0.0672	39.50	10
17	A_2		4.35	0.90	0.0644	14.77	5
17	B			0.80	0.0616	20.41	
17	C			0.90	0.0448	15.69	
18	A_1	Manure, 6 tons a	5.38	1.85	0.1232	49.60	28
18	A_2	year, corn con-	5.47	1.20	0.0896	14.91	10
18	B	tinuous		1.15	0.0700	18.57	
18	C			1.00	0.0420	18.29	
13	A_1	Corn, oats, wheat,	5.80	1.40	0.1064	10.6	7
13	A_2	clover, and timo-	4.55	1.00	0.0644	36.4	trace
13	B	thy 3 years		0.80	0.0560	28.96	
13	C			0.80	0.0392	18.15	

* No longer used as a check, lime accidentally applied in 1929. Plats 6, 7, and 10 started 1907; rotation corn-oats-clover.

† Plats 14 and 15 started 1897; rotation corn-wheat-clover.

C. Burnham made pH determinations, and L. Wursten, other determinations.

out irrigation, and a small increase in base exchange capacity appears to have occurred with these soil building treatments at least under rainfall farming. Crop data were previously reported (7).

Soil change in fertility plats at Astoria, Oregon. Further evidence of the effect of different soil treatments in maintaining soil fertility is found in the analytical data from certain plats of the Astoria, Oregon Branch Experiment Station. The soil is "Red shot" land developed under annual rainfall of approximately 100 inches so that the untreated soil is distinctly acid. The mineralization of manure applied or the application of lime has tended to decrease the acidity of these plats. These treatments have apparently increased the phosphate supplying power and, by aiding the growth of clover grown in a rotation with grain and roots on this land, have generally increased the nitrogen and organic

TABLE 2

Value of rotation, manure, and irrigation for maintaining fertility of soils

Bean rotation, irrigation field, Oregon Agricultural Experiment Station, Corvallis, Oregon, maintained 20 years; surface 0–7 inches.

TREATMENT	TOTAL NITROGEN	ORGANIC MATTER	BASE EXCHANGE CONTENT
	per cent	*per cent*	*m.e.: 100 gm.*
Irrigated:			
Beans continuous	0.165	2.70	7.92
Rotated	0.189	2.60	8.15
Rotated and manured	0.221	3.30	8.32
Dry:			
Beans continuous	0.147	2.20	6.46
Rotated	0.174	2.50	7.47
Rotated and manured	0.195	2.60	7.78

matter content. Both lime and manure have resulted in an increased base exchange capacity and the calcium has markedly increased the exchange calcium of the soil. No correction for calcium carbonate on base exchange content was made. Sulfur has not improved this acid soil and has apparently decreased its base exchange capacity (table 3).

Soil changes in fertility plats at Union, Oregon. In the second section of table 3 will be found chemical characteristics of soil samples representing fertility plats of the Union Branch Experiment Station. The soil is Catherine silt loam developed under arid conditions and is of the nature of meadow land, being affected by ground water and having a good native supply of organic matter. The soil is nearly neutral in reaction but tends to become slightly acid with continued use of sulfur. The manure and complete fertilizer on this soil have resulted in an increase in total nitrogen and in organic matter. The legumes respond well to the sulfur treatment on this soil with a resulting increase in nitrogen and organic matter. There has been a slight build-up

in base exchange content where manure or complete fertilizer has been regularly applied. With the sulfur treatment no increase, even possibly a slight decrease, in base exchange capacity is indicated.

Soil changes in fertility plats at Burns, Oregon. The third section of table 3 shows characteristics of plat soils from the Harney Branch Experiment Station

TABLE 3

Value of various treatments for maintaining soil fertility

PLATS	TREATMENT	pH	AVAILABLE PHOSPHORUS	TOTAL N	ORGANIC MATTER	BASE EXCHANGE CONTENT	EXCHANGEABLE		
							Ca	Mg	K
			lbs. ac.	*per cent*	*per cent*	*m.e.: 100 gm.*			
*Astoria, Oregon Branch Experiment Station fertility plats**									
1, 11, 21	Untreated	4.80	43	0.329	7.19	8.35	4.92	2.90	0.53
2, 12, 22	Manured	4.98	65	0.352	8.69	9.47	5.92	3.05	0.50
3	Limed	5.91	40	0.393	8.89	17.75	14.80	2.75	0.20
4	Manure and lime	5.72	70	0.378	6.96	14.00	10.65	2.95	0.40
6	Manure, lime, and plats	5.73	75	0.378	6.74	13.60	9.80	3.20	0.60
19	Sulfur	5.05	40	0.292	8.03	6.93	4.00	2.60	0.33
Union, Oregon Branch Experiment Station fertility plats†									
1, 5, 9	Untreated	6.8	†Doubtful	0.216	4.61	44.06	34.10	9.13	0.83
12	Manured	7,1	Medium	0.221	4.91	56.23	38.14	14.61	3.48
11	N, P, and K	7.1	Medium	0.224	4.58	50.51	34.86	14.70	0.95
7	Sulfur	6.5	Doubtful	0.229	4.71	43.97	33.00	9.66	1.31
2	Phosphorus	7.0	Medium	0.201	4.26	45.24	36.80	6.97	1.47
Harney Branch Experiment Station, Burns, Oregon‡									
2, 6	Untreated	7.58	116	0.239	5.04	32.19	19.70		
7	Manured	7.72	136	0.291	5.50	46.08	21.50		
10	N, P, and K	7.42	120	0.258	4.68	35.99	21.00		
4	Sulfur	7.35	112	0.241	5.18	36.21	21.00		

* Surface soils (0–7 inches), humid section—maintained 12 years.

† Semi-arid section—maintained 13 years (Ill. Test).

‡ Arid section—maintained 13 years.

located at Burns, Oregon, under an arid climate with about 8 inches annual precipitation. The soil is dark very fine sandy loam and faintly alkaline. Manure and complete fertilizer appear to have increased the available phosphate and total nitrogen content. The manure has increased the organic matter content. Both treatments appear to have increased the base exchange capacity (the manure very definitely so) and also the exchangeable calcium. Sulfur is moderately beneficial to legumes on this soil, although the irrigation

water, which contains some sulfates, also improves the reaction of this faintly alkaline soil so that a larger accumulation of organic matter and nitrogen may form.

Soil changes in plats under dry-farming at Moro, Oregon. Effects of various soil treatments on soils from the dry land branch experiment station at Moro, Oregon, are presented in table 4. The annual precipitation at this station is about 12 inches, and humification of grain straw is slow. It has been possible to raise field peas annually with a tendency toward increased yields. Alfalfa decays rather slowly under the conditions. It appears from the data in table 4 that humus building under such drouthy conditions is a slow process. The only definite progress indicated by the data results from the use over a 21-year period of green peas each 4 years in comparison with the standard alternation of wheat with fallow. Green peas appear to have increased organic matter,

TABLE 4

Effect of various treatments on soils from the branch experiment station, Moro, Oregon

Sampled June, 1932, by Powers and Oveson. Determinations from surface samples, 0–8 inches.

PLAT	TREATMENT	ORGANIC MATTER	TOTAL NITROGEN	BASE EXCHANGE CONTENT
		per cent	*per cent*	*m.e. per gm.*
1068	Check—Crop residue experiment	2.10	0.0868	0.1174
1067	Strawy manure each 2 years	1.80	0.0980	0.1316
1069	Straw returned, 9 years	1.80	0.0952	0.1318
783	Wheat-Fallow Check	1.60	0.0728	0.1140
785	Green peas each 4 years, 21 years	1.90	0.0756	0.1408
Check; wheat-fallow by alfalfa land		1.70	0.0784	0.1250
Alfalfa ground—New grain; 5 years alfalfa, plowed 4 years; 2 crops, 2 fallows		1.70	0.0756	0.1252

nitrogen, and base exchange capacity. This is in agreement with controlled plant house and laboratory studies (3).

Soil changes due to reclamation of alkali land, Vale, Oregon. Soil changes due to reclamation of black alkali land are indicated by the data from a few selected plats as shown in table 5. Sulfur used in combination with manure has been effective in reclaiming virgin black alkali land when provided with deep drainage and copious irrigation. A nearly perfect stand of alfalfa has been established, and normal yields are now obtained. The check plats given similar treatment in every way except for omission of fertilizer or chemicals are still almost devoid of vegetation. It will be noted that the organic matter content of the treated plats has been practically doubled and that this increase is traceable to a depth of 20 inches in some cases. The nitrogen content has been increased from 50 to 100 per cent in the surface soil and has been definitely increased to the 20-inch level. Substantial increase in base exchange capacity is also indicated. The data reported elsewhere show that the char-

acter of the exchange bases has changed so that some 80 or 85 per cent of the exchange sodium has been replaced by exchange calcium, and the soil restored from a hard sodium clay to a friable fertile calcium clay.

TABLE 5

Soil changes due to reclamation of black alkali land, Vale, Oregon experiment field

Sampled fall, 1932—Maintained 11 years

PLAT	DEPTH	TREATMENT	ORGANIC MATTER	TOTAL NITROGEN	BASE EXCHANGE CONTENT
	inches		*per cent*	*per cent*	*m.e.: 100 gm.*
A	0–5	S, 1.5 tons and	2.35	0.0728	16.75
	5–20	manure, 10 tons,	1.00	0.0336	19.27
	20–40	first 6 years	0.80	0.0280	23.30
B	0–5	Check	1.33	0.0532	14.99
	5–20		1.00	0.0280	22.54
	20–40		1.00	0.0336	25.65
C	0–5	S, 0.75 tons and	2.75	0.0616	17.70
	5–20	manure 8 tons,	0.87	0.0308	20.42
	20–40	first 5 years	1.00	0.0280	23.24
E	0–5	Check	1.49	0.0392	5.31
	5–20		1.00	0.0224	16.19
	20–40		1.00	0.0308	24.88

DISCUSSION

Formerly Gedroiz (2) held that if the base exchange capacity of a soil became greatly reduced it could scarcely be restored. Recent discoveries (6) of the great base exchange capacity of the organic fraction of soils and of the effect of lime or a slight alkalinity in increasing absorptive capacity of the soil, however, betoken good progress in the restoration, or methods of conservation, of a base supplying power of a soil. Recent studies indicate more clearly the stable nature of the black earths where the given supply of organic matter is stabilized by the calcareous nature of the soil as well as by the continental semi-arid climatic conditions.

The studies herein reported indicate that the constructive humus building soil treatments, the employment of which in long-time field experiments has been associated with maintenance of good crop yields (7), have also resulted in increases in soil nitrogen, organic matter, and base exchange content. Fertilizers, where helpful to growth, have assisted in this humus building process. Livestock farming may aid maintenance of organic matter, whereas cultivation or uncontrolled grazing may lead to physical deterioration or increased erosion. The tendency for soil to deteriorate is most marked in humid climates where liming and perhaps mineral fertilizers may be necessary for success with legumes

in crop rotation. Further indications are that soil building along these lines is more readily accomplished in calcareous arid or semi-arid irrigated soil of medium texture and in a fairly cool climate. In an intimate neutral mixture of clay and humic colloids surface condensation may occur. Possibly molecular rearrangements involving splitting of double valence bonds may take place or addition compounds form between the clay acidoid and the ligno-protein nuclei, thus stabilizing or increasing base exchange capacity (4).

Russell (8) recognizes four sets of conditions: (*a*) Losses exceed returns with podzolization, (*b*) losses about balance returns in the brownearths, (*c*) losses are small or negligible with semi-arid grass lands in the stable black earth region, and (*d*) rainfall is insufficient for much growth, little humus is formed, and salts remain. Increase from maintenance of active nitrogenous organic matter may be expected to favor nitrification and sulfofication processes and even increase the phosphate supplying power of the soil. Increasing anion concentration under conditions favorable for activity of beneficial microörganisms of the soil should in turn result in bringing and holding nutrient bases in the soil solution, especially if a good supply of readily ionizable or exchange bases is present. Irrigation water may either increase or decrease the supply of soil nutrients, depending upon the quantity used and the composition of the water. Where only sufficient water is added to saturate the soil without drainage, plant food remains. The extent of leaching has been reported by Scofield (9) at the Grand Canyon Gaging Station for 5 years ending 1930, where the annual discharge has been 15,700,000 acre feet, carrying 12,000,000 tons of dissolved salts. Scofield discusses the possible base exchange reactions occurring in soil due to irrigation, particularly the replacement of earthy bases by alkaline bases, which are contained in some irrigation water with the consequent impairment of the physical conditions of the soil. Soil deterioration is more likely to result in a period of depression. Fortunately the increase from a humus building crop rotation comes with little or no cash outlay and frequently results in a decreased unit cost of production.

SUMMARY

A study has been made of certain chemical characteristics of soil samples collected from some of the oldest experimental plats of Oregon and of the eastern states. The long-continued use of manure, crop rotation with legumes, or liming in the case of acid soils has been associated with a definite increase in total nitrogen, soil organic matter, and base exchange content. It appears that the build-up or conservation of the soil's nutrient supplying power is more readily accomplished in the calcareous irrigated soils under a fairly cool climate. The results of the study should be of value in improving soils, in conserving soil fertility, or in guarding against soil degeneration.

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PHOSPHATE FIXATION AND PENETRATION IN SOILS[1]

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Experience has shown that many soils, particularly those of the lateritic group, although very low in readily available phosphorus, give little response in crop yields when soluble phosphate is applied. Because of these results, the conclusion has often been reached that these soils are not deficient in readily available phosphorus. Experimental work indicates that this lack of response may be due to a rapid fixation of the applied phosphorus into forms that are difficultly available to plants.

Ford (1, 2) studied the fixation and availability of applied phosphates, and found a tendency in certain soils toward a lack of response from phosphate fertilization, due to a change in the form of the applied phosphorus. His work, together with that of others, indicates that certain iron and aluminum minerals in the soil are largely responsible for this change. The reader is referred to his work for a review of the literature. A brief summary of the salient facts on the fixation of phosphorus in soils follows.

THEORY OF PHOSPHATE FIXATION

Phosphate fixation in soils may be purely chemical, the phosphorus taking the form of slightly soluble mineral phosphates, or it may be of a biological nature in which the phosphorus exists, first as living and later as inanimate organic or micro-organic material. This latter form of fixation is involved in the biological balance (4), and is very important in its relation to phosphorus availability to plants, but will not be considered further at this time.

On the basis of previous work, it appears that phosphorus is fixed in the soil largely through reactions with active calcium, iron, or aluminum. In this relation, these elements may be regarded as active when they unite chemically with phosphorus. Active calcium may be present in the soil in the exchangeable form, as calcium carbonate, or as any soluble calcium salt. Active iron and aluminum are believed to exist in the soil largely in the forms of the hydrated oxides. Although aluminum may be found in the solutions of mature soils, iron is rarely present in this way, except in mere traces. It is not necessary, however, for these

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[1] Contribution from the department of soils, University of Wisconsin, Madison, Wisconsin. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

After this paper was written, work was reported by V. L. Weiser (Fixation and penetration of phosphates in Vermont soils. Vt. Agr. Exp. Sta. Bul. 356), which confirms with soils from Vermont the findings reported in this paper. For an excellent review of the literature, the reader is referred to this work of Weiser.

[2] The writer is appreciative of the kindly cooperation of Prof. E. Truog in connection with this study.

elements to be in solution in order to react with soluble phosphates to form phosphates of low solubilities, since the natural hydrated oxides react directly, as shown by Ford (2).

Figure 1 shows graphically how varying proportions of active calcium, iron, and aluminum in soils may determine the amounts of the different phosphates formed through fixation, and hence the availability of the applied phosphate. This figure is based upon general information and not upon specific data, and is presented to illustrate the relation of these elements to the fixation of phosphorus in soils.

With sufficient calcium and relatively small amounts of active iron and aluminum in the soil, most of the phosphorus is fixed as tricalcium phosphate. This phosphate is only slightly soluble in water, but easily soluble in 0.002 *N* sulfuric acid buffered to pH 3.[3] In this form, the phosphorus may be regarded as readily available for plant growth. Soils that fix phosphorus in this form are usually basic or only very slightly acid, and apparently contain relatively low amounts of active iron or aluminum. Miami silt loam (fig. 1, B) is a soil showing this type of fixation.

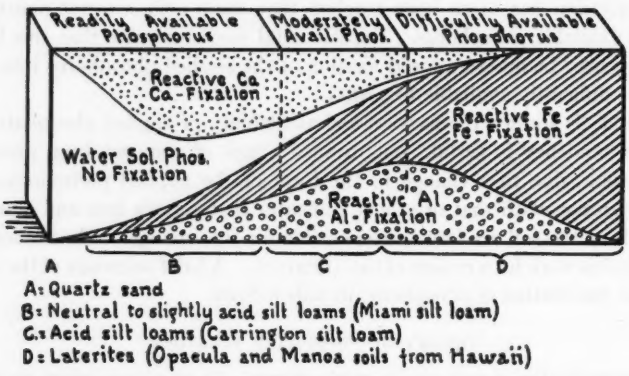

FIG. 1. THE RELATION OF CALCIUM AND ACTIVE ALUMINUM AND IRON TO THE FORM AND AVAILABILITY OF APPLIED PHOSPHORUS FIXED IN THE SOIL

When active calcium is low and the active agent of fixation consists largely of iron with some aluminum, the phosphorus is fixed, for the most part, as iron phosphates. The normal iron phosphate and the basic iron and aluminum phosphates are only very slightly soluble in 0.002 *N* sulfuric acid, and, for this reason, these three forms of fixed phosphorus may be regarded as difficultly available. Laterites (fig. 1, D) usually exhibit this type of fixation because of the high content of hydrated iron and aluminum oxides.

The normal aluminum phosphate is sufficiently soluble in 0.002 *N* sulfuric acid to justify its being classed as moderately available. Fixation in this form generally takes place in soils that are distinctly acid, because of the tendency of these soils to contain soluble aluminum, and also because the low pH value favors the combination of phosphorus with aluminum rather than with calcium. Carrington silt loam (fig. 1, C) tends toward this type of fixation.

In soils with little or no active calcium, iron, or aluminum, the soluble phosphate tends to remain soluble and be subject to leaching. This condition, however, is only encountered in soils made up largely of quartz (fig. 1, A).

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[3] This 0.002 *N* sulfuric acid is buffered to pH 3 with potassium sulfate, and in this paper will be referred to as "0.002 *N* sulfuric acid."

It is thought that magnesium, manganese, and titanium may also function to some extent in the fixation of phosphorus, but their importance is questionable, except in very special cases.

Fixed phosphorus may then be regarded as falling into three groups; (*a*) the readily available [$Ca_3(PO_4)_2$], (*b*) the moderately available [$AlPO_4$], and (*c*) the difficultly available forms [$FePO_4$, $Al_3(OH)_3PO_4$, $Fe_2(OH)_3PO_4$].

Considering these facts, it was the purpose of this work to determine more definitely the nature of the phosphates formed in soils through fixation of applied soluble phosphates.

EXPERIMENTAL

Nature of the phosphates formed through fixation in the soil

The nature of the phosphates formed by fixation in soils was studied by comparing the solubilities or the rate of solution curves of different phosphates with those of the phosphates in soils before and after treatment with soluble phosphate. The data for these curves were obtained by leaching small amounts of the different materials with 0.002 *N* sulfuric acid. The leaching was done at a uniform rate of 25 cc. an hour. The method of determining readily available phosphorus was essentially that of Truog (7). The data for the known phosphates and those for soils are shown graphically in figure 2.

Solubility of known phosphates. The curves in figure 2 were plotted from data taken at intervals of 2 hours, and represent the rate at which phosphorus was dissolved from the various materials during a 12-hour period. The area under the curve for any substance represents the amount of phosphorus dissolved from that substance in any given unit of time, and in this respect each graph is a two dimensional figure.

When tricalcium phosphate or finely ground rock phosphate was subjected to this leaching process, there was complete solution of the phosphorus in the first 50-cc. portion, or in 2 hours' time. If the amount of phosphorus going into solution in any one unit of the 2-hour period is proportional to the amount still undissolved, then the straight line of curve 1 represents the rate of solution of tricalcium phosphate or finely ground rock phosphate when leached in this way. The curves for tricalcium phosphate and for rock phosphate coincide, and in each case the total of 500 p.p.m. of applied phosphorus was recovered.

Aluminum phosphate dissolves much more slowly than calcium phosphate, and, with the exception of the first period, the curve for this substance (curve 2) is nearly a straight line, somewhat parallel with the base line, but slowly approaching it as leaching proceeds. The curve for ferric phosphate (curve 3) is much nearer the base line and practically parallel with it, whereas the curve for dufrenite [$Fe_2(OH)_3PO_4$] (curve 4) practically coincides with the base line. With the exception of tricalcium phosphate and rock phosphate, each phosphate shows a distinctive solubility curve. With both the iron and aluminum phosphates, the first unit of time gave a greater solubility than the remainder of the curve would seem to indicate. This leads one to suspect either that perhaps both of these substances contained impurities as easily

soluble phosphate, or that hydrolysis took place much more rapidly during this period. The former view seems probably the more logical.

Solubility of soil phosphates, and nature of fixed phosphorus. Miami silt loam, a glacial soil from Wisconsin, and the Opaeula soil, a laterite from Waialua, Hawaii, were each treated with 500 p.p.m. of phosphorus as monocalcium phosphate, and allowed to stand for 2 days under optimum conditions of moisture and temperature. The soils were then dried and both the treated and untreated soils were leached with 0.002 *N* sulfuric acid. The results are shown in the second part of figure 2.

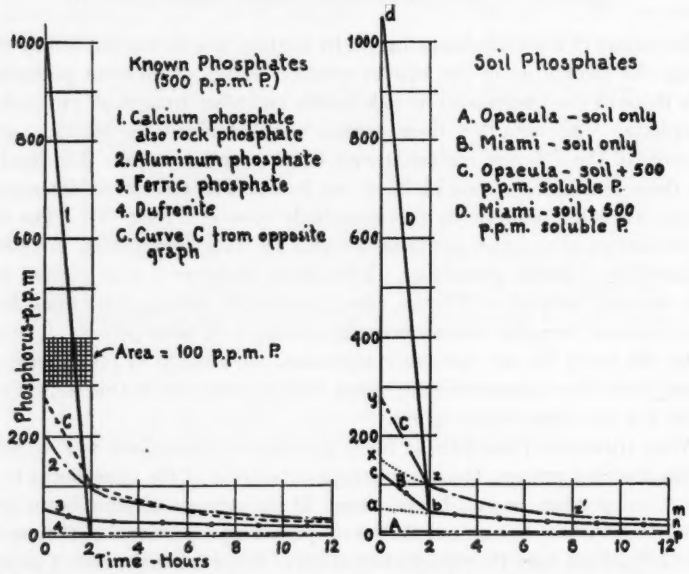

Known Phosphates
(500 p.p.m. P.)
1. Calcium phosphate also rock phosphate
2. Aluminum phosphate
3. Ferric phosphate
4. Dufrenite
C. Curve C from opposite graph
Area = 100 p.p.m. P
Phosphorus-p.p.m.
Time - Hours
Soil Phosphates
A. Opaeula - soil only
B. Miami - soil only
C. Opaeula - soil + 500 p.p.m. soluble P.
D. Miami - soil + 500 p.p.m. soluble P.

FIG. 2. RATE OF SOLUTION OR SOLUBILITY CURVES FOR KNOWN PHOSPHATES AND FOR THE NATIVE AND APPLIED PHOSPHATES IN SOILS (0.002 *N* H_2SO_4 OF pH 3)

Curve A for the untreated Opaeula soil, very nearly coincides with the base line, which indicates that the form of the native phosphorus in this soil is similar to that in dufrenite. The form of the curve (B) for Miami silt loam, indicates that the phosphorus is present in this soil in at least three forms,—calcium, aluminum, and iron phosphates.

The recovery of phosphorus from the treated soils is shown in curves C and D. The treated Miami silt loam (curve D) gives a solubility curve during the first 2 hours similar to that for tricalcium phosphate or rock phosphate, and at point *b* this curve coincides with curve B and follows it to the end of the 12-hour period. This behavior may be interpreted to mean that the applied phosphate has been fixed in this soil largely as a calcium phosphate and is almost

entirely removed at the end of 2 hours' leaching. This fact, together with the coincidence of the two curves between *b* and *n*, shows that little or no iron and very little aluminum phosphate of any kind were formed. If the curve *bn* is projected to meet the ordinate, the triangle *abc* represents approximately the amount of readily available phosphorus in Miami silt loam before treatment, and the triangle *abd*, the amount after treatment with 500 p.p.m. of phosphorus as monocalcium phosphate. If there had been no fixation of the applied phosphorus in difficultly available form, the area of the triangle *abd* minus the area of the triangle *abc* should equal the 500 p.p.m. of phosphorus applied, and with the Miami silt loam used, this is true to the extent of about 95 per cent.

The results with the Opaeula soil were entirely different, as is shown in curve C. First, there is much less phosphorus extracted during the first 2 hours than from the treated Miami silt loam, and, after this, there is no coincidence of curve C with the corresponding parts of curve A. The spread between *op* and *zm* indicates that a considerable quantity of the phosphorus applied to the Opaeula soil has been changed to slightly soluble iron and aluminum phosphates. The arc *zz'* indicates aluminum phosphate, and the almost straight line *z'm*, iron phosphate. If the arc *zz'* is projected to meet the ordinate, the triangle *xyz* represents the readily available phosphorus which, in this case, is probably made up of both calcium and aluminum phosphates. The area *yzmpo* roughly represents the amount of the applied phosphorus recovered as calcium, aluminum, and normal iron phosphates, and the difference between this quantity and the 500 p.p.m. of applied phosphorus is that quantity which has gone over to the iron and basic aluminum phosphates.

If curve C is superimposed upon the left half of figure 2, its partial similarity to curves 1, 2, and 3 can be easily seen. This is an indication that it is a composite of these three curves, and that its form comes from varying amounts of these phosphates or phosphates of similar solubility.

Additional data obtained in a similar manner to that illustrated in figure 2 were obtained for the two soils already reported, and for Carrington silt loam, another glacial soil from Wisconsin, and the Manoa soil, another laterite from the upper Manoa valley, Hawaii. In this case the soils were treated with 400 p.p.m. of phosphorus in solution as monocalcium phosphate, boiled to dryness, and then leached in the way previously described. The amounts of the different fixed phosphates were then calculated from the solubility curves. Table 1 gives these data.

The phosphorus given in the last column of table 1 includes all of the calcium and a part of the more easily soluble aluminum and iron phosphates, and was obtained by a single 30-minute extraction with 0.002 *N* sulfuric acid, and a ratio of soil to solution of 1 to 400. Figure 3 shows graphically the relative amounts of the different phosphates formed by fixation in each of the four soils studied.

In the two glacial soils, a relatively large amount of the applied phosphorus is held in the calcium form. As the pH value of the soil becomes less, the

phosphorus fixed as calcium phosphate decreases, and the iron and aluminum or the difficultly available phosphates increase. In the Hawaiian laterites, the

TABLE 1

Relative amounts of different phosphates formed and the amounts of readily available phosphorus recovered from soluble phosphates applied to soils

SOIL	pH	APPLIED PHOSPHORUS			
		Changed to forms indicated			Recovered as readily available by 0.002 *N* H_2SO_4 extraction
		Calcium phosphate (Readily available)	Alumium and iron phosphates (Moderately available)	Basic aluminum and iron phosphates (Difficultly available)	
		per cent	*per cent*	*per cent*	*per cent*
Miami silt loam	6.66	83.5	7.5	9.0	85.5
Carrington silt loam	5.67	52.0	26.7	21.3	68.7
Hawaiian, Opaeula	6.09	2.0	27.0	71.0	19.5
Hawaiian, Manoa	5.40	0.0	Not determined	Not determined	8.7

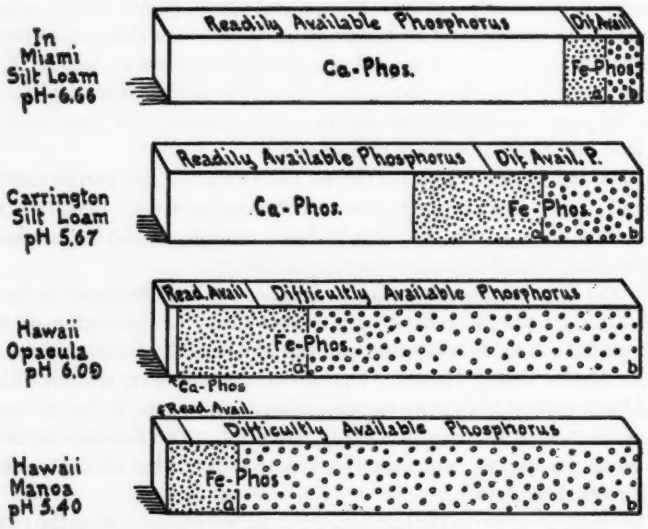


FIG. 3. THE RELATIVE AMOUNTS AND AVAILABILITY OF THE PHOSPHATES FORMED IN DIFFERENT SOILS FROM APPLIED SOLUBLE PHOSPHATE

phosphates of iron and aluminum or the difficultly available forms make up by far the greater part of the fixed phosphorus. Although the pH value of the soil is an important factor in determining the form in which the phosphorus is

fixed in a soil, this factor may be largely overshadowed by the presence of large amounts of hydrated oxides of iron or aluminum.

In figure 3, the part designated as iron phosphate includes not only the iron but also the aluminum phosphates. The *a* fraction may be spoken of as being moderately available, and is perhaps made up largely of aluminum phosphate together with the more soluble fraction of the iron phosphate. The possibility of phosphorus in organic combination making up a portion of this fraction is eliminated by the conditions under which the soluble phosphate was fixed in the soil. The *b* fraction is made up largely of the basic phosphates of iron with some basic aluminum phosphate and some normal iron phosphate. This division point shifts as the equilibrium changes, with the tendency for the more difficultly available phosphates to increase.

Penetration of applied phosphates

Aside from the form, the position in which the applied phosphorus is fixed in the soil is also important. In order to be available for plant growth, the phosphorus must be within the root zone. Soils vary greatly as to the extent to which they permit the penetration of soluble phosphates. The extent of penetration is governed largely by the nature and the amounts of the materials in the soil which are capable of precipitating the soluble phosphate.

The four soils previously described were used for a study of positional fixation. Tubes about 2 inches in diameter were filled with these soils to a depth of 5 inches. Enough phosphorus (42 mgm.) in solution as monocalcium phosphate to make up approximately 225 p.p.m., based on the entire soil mass, was placed on the surface of the soil, and 6 inches of irrigation water was applied. The phosphorus leaching through the soil and the amounts of readily available fixed phosphorus in the various strata of these soil columns were determined. In table 2 are given the amounts of the applied phosphorus recovered from the soil in readily available form and also the place where it was found after fixation. Figure 4 shows in graphic form the penetration of phosphorus into each of the four soils studied.

Figure 4 is constructed on a two dimensional basis, and the amounts of the different phosphates represented are proportional to the areas shown. Of the phosphorus fixed in the soil, the position and quantity of the readily available forms only are shown, and it is assumed that the difficultly available phosphorus is fixed approximately in the same position.

The greatest penetration of phosphorus in these four soils occurred in the Miami silt loam and the least in the two Hawaiian laterites, with the Carrington silt loam falling intermediate. The water-soluble phosphorus passing through 5 inches of soil also stands in this same relationship. The Miami silt loam apparently contains relatively little active iron or aluminum and not more than a trace of calcium carbonate. With these factors balanced in this way, this soil is almost ideal for the effective use of soluble phosphates. There is very little difficultly available phosphate formed, and there is sufficient ex-

TABLE 2

Readily available phosphorus recovered at different depths and from drainage water, from soluble phosphate applied with irrigation to the surface of soils

	READILY AVAILABLE PHOSPHORUS RECOVERED FROM 42 MGM. OF APPLIED PHOSPHORUS			
	Miami silt loam	Carrington silt loam	Hawaiian Opaeula	Hawaiian Manoa
	mgm.	*mgm.*	*mgm.*	*mgm.*
Recovered from soil				
First half inch	4.02	6.82	13.20	2.94
Second half inch	2.42	2.62	1.07	0.00
Total first inch	6.44	9.44	14.27	2.94
Second inch	4.84	5.12	0.00	0.00
Third inch	4.84	4.61	0.00	0.00
Fourth inch	4.84	3.54	0.00	0.00
Fifth inch	4.84	2.94	0.00	0.00
Total recovered from soil	25.80	25.69	14.27	2.94
Recovered in the drainage water	15.50	2.30	0.00	0.00
	per cent	*per cent*	*per cent*	*per cent*
Total recovered in readily available form	98.4	66.6	34.0	7.0

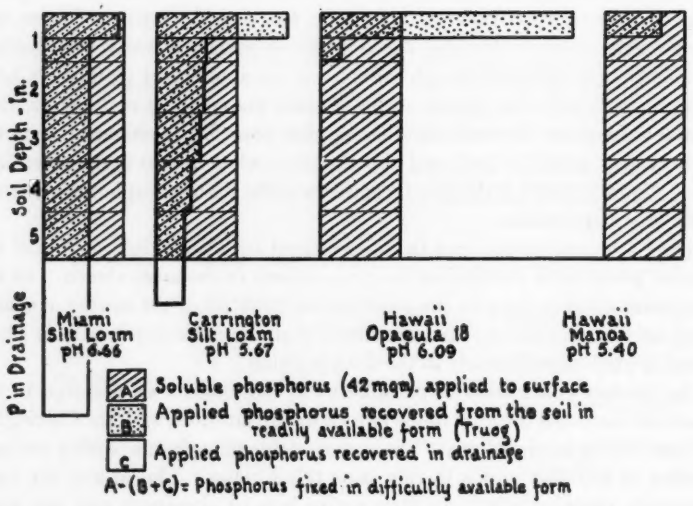


FIG. 4. THE DOWNWARD PENETRATION OF PHOSPHORUS AND ITS RECOVERY IN READILY AVAILABLE FORM (TRUOG) WHEN SOLUBLE PHOSPHORUS IS APPLIED WITH IRRIGATION TO THE SURFACE OF 5-INCH DEPTHS OF DIFFERENT SOILS

changeable calcium present to prevent excess leaching, and, at the same time, not enough calcium carbonate to hold the phosphorus in the immediate surface soil. As a result, a good positional fixation is obtained.

As soils become more acid and the pH value drops below 6.5, there is a greater tendency for the iron and aluminum to unite with the phosphorus. This is due, in part, to a tendency for a greater amount of active iron and especially aluminum to be present in the more acid soils, and, in part, to a smaller amount of calcium in the fixation equilibrium, thus giving the iron and aluminum a better chance to react with the phosphorus. This appears to be true of the Carrington silt loam which has a pH value of 5.6. As compared with the Miami silt loam, there is a very much greater fixation of phosphorus in the difficultly available form, and, at the same time, a very much greater tendency to hold the readily available phosphorus in the surface soil.

If the soil should contain a considerable amount of calcium carbonate, the phosphorus would be held largely in the immediate surface soil as tri-calcium phosphate. In this case the form of the fixed phosphorus may be satisfactory from a purely chemical standpoint, but the excess of lime present and the nearness of the fixed phosphorus to the surface of the soil render this type of fixation less desirable.

The laterites considered in figure 4 allow only a very slight penetration of the soluble phosphate (even though the physical condition is ideal for its movement). In neither laterite has the soluble phosphate penetrated more than half an inch. At the same time, there is a fixation in the difficultly available form of from 66 to 93 per cent of the applied phosphorus. As a rule, these soils contain a very low amount of dializable iron or aluminum, but apparently large quantities of hydrated oxides of these elements which react with soluble phosphate and not only hold it in the least soluble form, but also very near the surface. Compared with the glacial soils shown, the two laterites from Hawaii may truly be called high fixing soils, not only with respect to the form, but also to the position in which the phosphorus is fixed.

Stephenson and Chapman (6) found a varying penetration in the field, depending upon the kind of soil, the amount of the applied phosphorus, and the manner of its application. However, as a rule, it is generally thought that under actual field conditions the same soil will allow much less penetration than under ideal laboratory conditions, especially where the application in the laboratory is made in solution and followed immediately with large amounts of irrigation water.

DISCUSSION

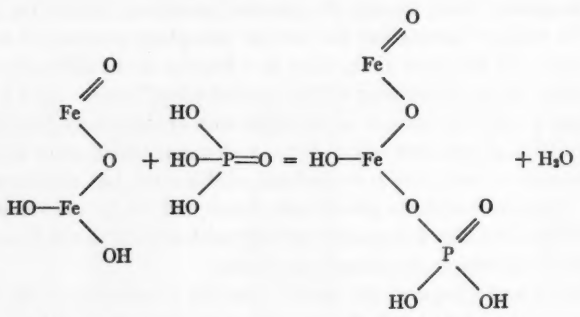
The work of Gaarder (3) indicates that the pH value of the soil is an important factor influencing the forms in which phosphates are fixed in soils. He reports that the amount of phosphorus going into solution from calcium phosphate at pH 6.5 is only about one-third that from normal aluminum phosphate and only about one-fourth that from normal iron phosphate, whereas at pH 5.5

the phosphorus from calcium phosphate has from 3 to 5 times the solubility of that in the iron and aluminum forms. Based upon these facts, it appears that a lower pH value in a soil not only increases the solubility of the phosphorus from calcium phosphate but at the same time reduces the amount of calcium in the mass action equilibrium with phosphorus, both of which tend to increase the amount of phosphorus fixed in difficultly available form.

The fixation of phosphorus as iron or aluminum phosphates does not necessarily depend upon iron or aluminum in the soil solution, but rather upon the state of hydration of their oxides. If the iron oxide is fully hydrated, the normal phosphate may be formed:

$$2Fe(OH)_3 + 2H_3PO_4 \rightleftarrows 2FePO_4 + 6H_2O.$$

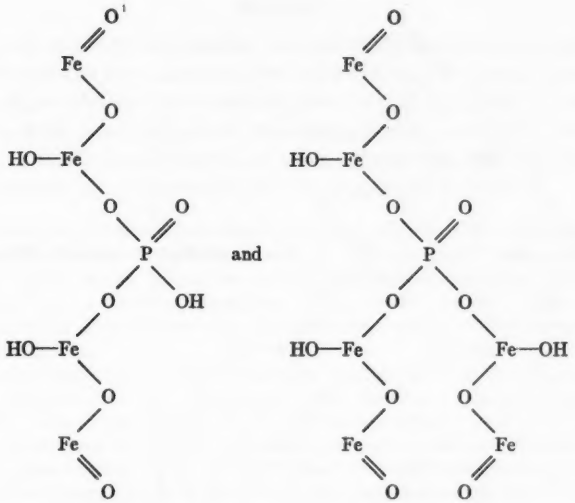
There is some question, however, as to the extent of this reaction, because of the instability of the tri-hydrated iron oxide and its tendency to change to the monohydrate, which is the more stable form. Ford (2) has shown that dehydrated iron oxide (hematite, Fe_2O_3) does not react with soluble phosphates, but that ferric oxide monohydrate (Goethite, $Fe_2O_3 \cdot H_2O$) is very reactive. The reaction seems to be one of simple addition, making it unnecessary for the iron to go into solution, and may be illustrated as follows:



Ferric oxide monohydrate (Goethite)

Ferric oxide monophosphate (dufrenite)

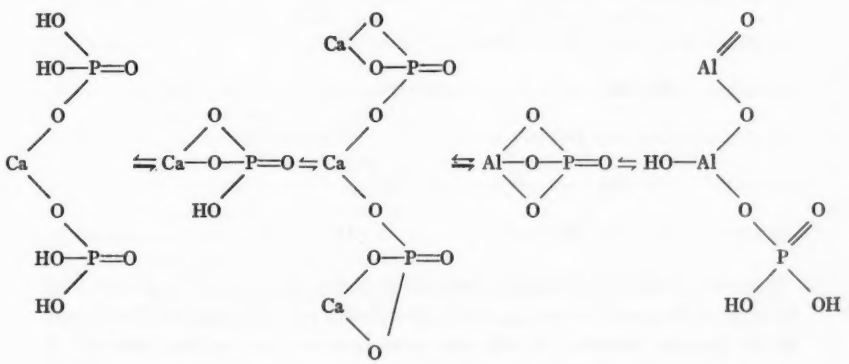
The work of Leahey (5) presents some evidence that there may be combinations of iron with phosphorus in which the ratio of ferric oxide to phosphoric acid (P_2O_5) is four to one or even six to one. This would suggest at once the di- and tri-ferric oxide combinations with phosphoric acid, which may be represented as follows:

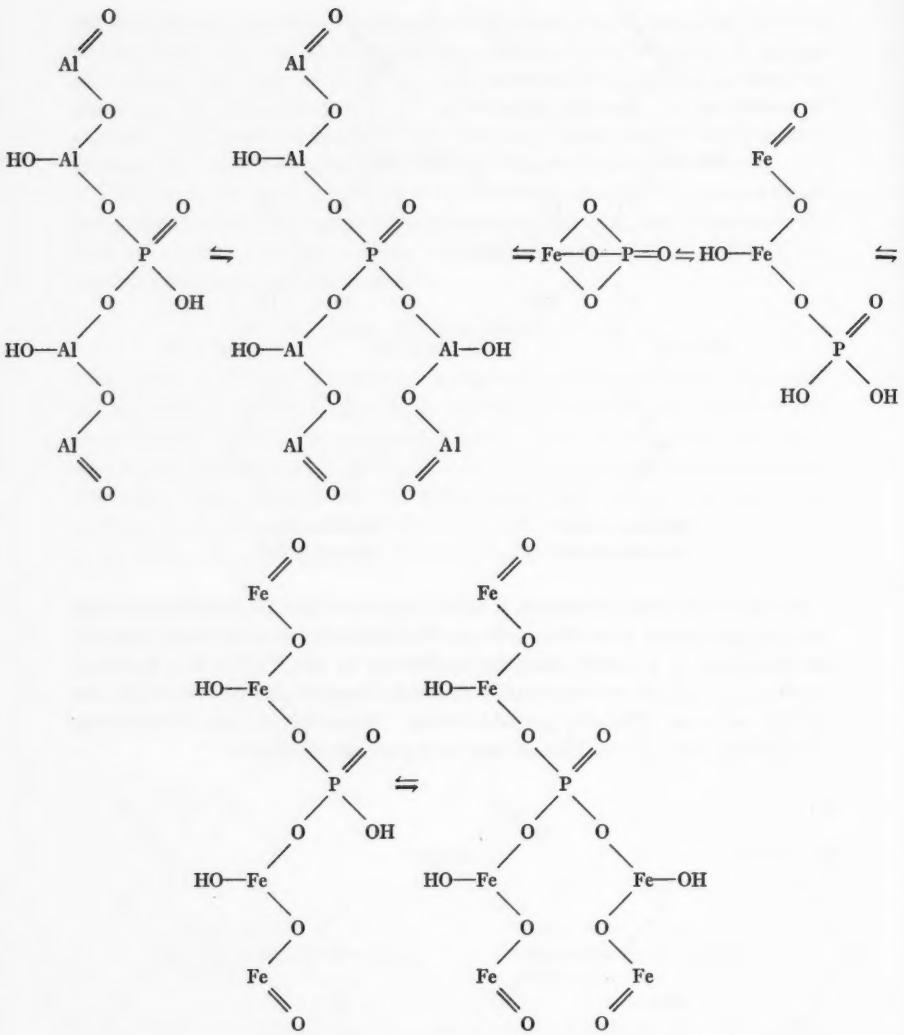


Di-ferric oxide monophosphate

Tri-ferric oxide monophosphate

In view of the facts presented, it seems logical to think of phosphate fixation in soils as a change from the soluble to the relatively insoluble forms, and the establishment of a slowly changing equilibrium to which there is a tendency for the more soluble or more readily available forms to change over to the less soluble and more difficultly available forms. Somewhat in order of decreasing availability, such an equilibrium may be expressed as follows:





Such an equilibrium is highly theoretical, and is shown only to give an idea of some of the possible ways in which phosphorus may be combined in the soil in the fixation process. It will also serve to show how varying amounts of active calcium or of the hydrated oxide of iron or aluminum in the soil may influence the extent and kind of fixation taking place.

SUMMARY

In this study of phosphate fixation, four rather different soils were used in an attempt to ascertain more definitely the nature of the compounds formed and the depth of penetration when a soluble phosphate was applied to these soils. The nature of the fixed phosphates was determined by means of comparative solubilities (in 0.002 *N* sulfuric acid) of known phosphates and those formed in soils through fixation. The results may be summarized as follows:

The predominating form in which soluble phosphate is fixed in the soil depends much upon the relative abundance in the soil of the different materials capable of fixing phosphorus. If the ratio of active calcium to active iron and aluminum is high, the fixation will be largely in the calcium form, and the fixed phosphorus will be readily available. If the reverse is true, the fixation will be largely as the iron and aluminum compounds of phosphorus, which are difficultly available.

On the basis of solubility, the soluble phosphate was fixed in the Miami silt loam largely as calcium phosphate. In the Carrington silt loam, which was more acid, less calcium phosphate and more iron and aluminum phosphates were formed. In the two Hawaiian laterites, the fixation was largely as the basic forms of iron and aluminum phosphates. Although the reaction and the presence of reactive calcium are important factors governing the forms of the fixed phosphorus, in the laterites these factors were overshadowed by the presence of very large quantities of the hydrated oxides of iron and aluminum.

The greatest phosphate penetration occurs in soils of neutral to slightly acid reaction which contain little active iron or aluminum. Either active calcium, iron, or aluminum inhibits the downward movement of phosphorus in soils in proportion to the amounts present and inversely proportional to the solubilities of their compounds with phosphorus. Of the soils studied, the slightly acid Miami silt loam with little active iron or aluminum gave maximum penetration, whereas the laterites, with large amounts of active iron and aluminum, allowed very little penetration, the phosphorus being fixed and held largely in difficultly available forms within a few millimeters of the point of application.

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THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE CULTURE SOLUTION UPON THE ABSORPTION AND ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN BY PEACH TREES GROWN IN SAND CULTURES[1]

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A comparison of various forms of nitrogen for the purpose of determining which form is the most satisfactory for plants in general, or for specific plants, has attracted considerable interest in the past. Recent investigations, however, have complicated considerably the problem of comparing different forms of nitrogen used for plant nutrition.

Thus the work of Stahl and Shive (23, 24) has shown that some plants may, during the early stages of their life cycle, absorb nearly all of their nitrogen in the ammonium form from nutrient solutions containing both ammonium and nitrate ions. However, as the plants increase in age, under the same set of experimental conditions, it was found that the rate of absorption of ammonium nitrogen decreases. The relative proportions of ammonium and nitrate nitrogen absorbed during various stages of the life cycle were found to vary considerably among different species. Pirschle (13), Tiedjens and Robbins (29), and Tiedjens and Blake (28), on the other hand, have shown recently that the ability of plants to grow in water or sand cultures containing either ammonium or nitrate nitrogen depends primarily upon the reaction of the nutrient solution used.

In the investigations reported herein, attempts have been made to study the influence of the hydrogen-ion concentration of the nutrient solution employed upon the absorption and assimilation of ammonium and nitrate nitrogen. Since herbaceous types of plants have usually been employed for absorption studies, it was considered desirable to use trees in these experiments. Preliminary work has shown that peach trees are well adapted for use in sand cultures, and that they grow rapidly and respond quickly to nutrient treatments. Accordingly, in the experiments described in the following pages, peach trees (*Prunus persica* Stokes) have been grown and studied in two series of treatments, one containing nitrogen only in the ammonium form and the other containing nitrogen only in the nitrate form.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

[2] It is a pleasure to acknowledge indebtedness to Dr. G. T. Nightingale for many helpful suggestions relating to methods of analysis, and to Prof. M. A. Blake for valued assistance in the selection of material.

EXPERIMENTAL METHODS

Plant material

Seedling peach trees were used in these studies in order to avoid variations in growth that may result from differences in the amounts of reserve foods present in older trees. It was recognized, of course, that the use of seedling trees might involve inherent variations in habit and vigor of growth, as well as possible differences in composition. As a result of peach breeding investigations conducted at the New Jersey Experiment Station for many years, the growth habits of seedlings from numerous varieties of peaches are well known. Accordingly, at the suggestion of Prof. M. A. Blake, seedlings grown from pits from an open pollinated Early Crawford tree were chosen. Seedlings from this variety are homozygous in many of their characters and are nearly uniform in their habits of growth. The method used for propagating the plants and handling them prior to the selection of a group of individuals for these experiments has been described in an earlier publication (1) and need not be repeated here.

For use in these studies, groups of uniform seedlings with tops about 15 cm. high were selected. They were transplanted into 2-gallon glazed porcelain percolators filled with carefully washed white quartz sand. These percolators were 24 cm. in diameter, 30 cm. in height, having perpendicular sides and a rounded bottom with a 2.5 cm. drain hole in the center, thus providing perfect drainage. Two plants were placed in each pot. The drain hole in the pot was covered with a watch glass which, in turn, was covered by a pad of glass wool. This arrangement prevented even the finest sand particles from washing through the hole, and at the same time it permitted free drainage.

Culture solutions

For the purpose of these studies, it was necessary to use two different series of nutrient solutions: one having all of its nitrogen in the ammonium form, and the other having all of its nitrogen in the nitrate form. Moreover, in order to permit an approximate comparison of the absorption and assimilation of these two forms of nitrogen, it was considered desirable to use culture solutions all of which contained, in so far as possible, the same concentrations of essential elements, especially nitrogen. The importance of such a balance is emphasized by the work of Prianischnikow (16, 17, 18), and of Holley, Pickett, and Dulin (5). It is not possible, however, to prepare two solutions having identical concentrations of nitrogen, one containing the nitrate ion and the other the ammonium ion, and still have identical concentrations of all other ions. Accordingly, the amounts of magnesium and sulfate ions were varied slightly, since it has been shown (1) that peach trees grow well over a very wide range in concentrations of these two ions. The similarity with respect to the ionic composition of the two series of solutions was changed further by the amounts of normal potassium hydroxide and hydrochloric acid added to obtain the desired hydrogen-ion concentrations.

Peach trees have been found (1) to grow especially well in sand cultures when supplied continuously with culture solutions containing medium to high concentrations of potassium and phosphate ions. A high concentration of phosphate in a solution having a low hydrogen-ion concentration, however, brings about an undesirable precipitation of calcium and magnesium. Therefore Number 12, or the $T_3R_1C_3$ solution, of the ammonium sulfate series of Jones and Shive (7) was modified as shown in table 1 to meet the requirements of this investigation. Each of the modified solutions has an osmotic concentration of approximately 0.9 atmosphere.

Boron and manganese were supplied to all cultures by the addition of a solution of boric acid and manganese sulfate directly to the culture solutions at the rate of 0.5 p.p.m. of each of these elements. Normal HCl and KOH were used to adjust the solutions to the desired pH value. These culture solutions were supplied to the plants continuously by the method of Shive and Stahl (22).

TABLE 1

Composition of nutrient solutions

	PARTIAL VOLUME-MOLECULAR CONCENTRATION	
	Ammonium series	Nitrate series
KH_2PO_4	.....	0.00633
K_2SO_4	0.00316	.....
$CaH_4(PO_4)_2$	0.00317	.....
$Ca(NO_3)_2$	.....	0.00318
$Mg(NO_3)_2$	.....	0.00120
$MgSO_4$	0.00711	0.00711
NH_4OH	0.00876	.....

Absorption tests

The sand culture method of growing plants does not lend itself so readily to determination of the rates of ion absorption by plants as does the solution culture method; nor is it possible to determine these rates for sand culture plants with the same degree of accuracy as it is for plants grown in solution culture. This is due, of course, to the fact that, first, the plants grown in a sand culture cannot be removed from the sand and transferred to a separate and special culture vessel for the purpose of making a test; and, second, the culture solution used for the test cannot be removed quantitatively for chemical analysis from the sand in which the plants are rooted. Thus, an approach to accuracy in making absorption tests with plants grown in sand cultures appears to depend largely upon the use of a method which permits precisely similar treatment of every culture.

The method which was finally adopted for use in these experiments is described as follows: A rubber stopper containing a short length of glass tubing was inserted tightly into the drain hole of each culture vessel used for absorp-

tion tests. By means of a short length of rubber tubing, a culture was connected to a 1-liter suction flask, and most of the free nutrient solution held in the sand was withdrawn by suction. Following this, the sand was washed with a liter of distilled water added in four applications. The pot was allowed to drain for 15 minutes, and then suction was applied until only an occasional drop of liquid was withdrawn.

Exactly 1 liter of the respective test solution was supplied to the culture by the method of continuous solution renewal mentioned previously. All solution that leached through the culture during the test period was collected in a carefully cleaned granite-ware pan placed beneath the drain. The rate at which the solution was supplied during the test always corresponded to the rate at which the solution was supplied per day to that particular culture. Thus, if the culture had been receiving 4 liters of solution daily, the absorption interval was 6 hours.

Immediately after the termination of the absorption interval, the free nutrient solution held in the sand was withdrawn by suction, just as the solution was withdrawn preparatory to the test. This solution was added to the leachings. The sand was then washed with 1 liter of distilled water as before and allowed to drain for 15 minutes. The excess wash water was then withdrawn by suction as before and added to the leachings. The pan, suction flask, and rubber tubing were washed, and the wash water, together with the leachings, was made to comprise a volume of exactly 2 liters. The amount of moisture transpired by the plants during the test was usually far greater than the amount of water required to wash the apparatus.

By a comparison of the amount of nitrogen found in an aliquot of 2 liters of solution that passed through the cultures with that found in an equivalent aliquot of the initial solution, the approximate amount absorbed by the plants during the test was determined.

The treatments given the cultures before and after the test solution was applied were made as nearly alike as possible. Special care was taken to handle in precisely the same manner all cultures used in a given test. Of course, it is to be assumed that, at best, the method can give only an approximate measure of the nitrogen absorbed by the plants from the test solutions passing in a given interval of time through the sand in which the plants were rooted. Nevertheless, these approximate absorption rates of the various cultures should be comparable one with another.

In order to study the absorption of nitrogen by cotton seedlings grown in sand cultures, Naftel (9) used a method somewhat similar to the one described. He used only a kilogram of sand per culture, however, and after washing it with 500 cc. of distilled water to remove the nutrient solution, allowed it to drain overnight. By such treatment, he undoubtedly washed the free nutrient solution from the sand very thoroughly and obtained a satisfactory indication of the amounts of nitrogen absorbed by plants in the different treatments. Nevertheless, he subjected his plants to drastic changes in osmotic pressure

over comparatively long periods of time. It is probable that the method employed for these experiments with peaches avoided, or at least minimized, any injurious effects which this might have had upon the plants, and also that it minimized any tendency toward the excretion of ions from the roots.

EXPERIMENTAL RESULTS

Two sets of experiments, extending over a period of 2 successive years, were carried out. These will be referred to as the "1931 experiments" and the "1932 experiments," and will be treated separately. The 1931 experiments must be regarded in the nature of a preliminary investigation only, but since they brought out some important points, the data obtained will be presented and discussed in some detail. For the sake of brevity in considering results and the data to be presented, reference will be made to the various pH treatments and to the plants grown under them by the use of simple abbreviated expressions. Thus the pH-4 treatments in the ammonium series will be referred to as the "NH_4-pH-4 treatments," and the plants grown under these treatments will be designated the "NH_4-pH-4 plants." The corresponding treatments in the nitrate series will be called the "NO_3-pH-4 treatments" and the plants will be designated the "NO_3-pH-4 plants." Other treatments and the plants grown under them will be designated by corresponding terms relating to the pH of the culture solution and the series in question. In all cases sufficient plant material was produced under each treatment, both in the ammonium and in the nitrate series, to furnish adequate samples for chemical analyses for the purpose of determining the distribution of the nitrogen fractions in the plants.

1931 experiments

On June 7, a sufficient number of uniform seedlings with tops about 15 cm. high were selected and transplanted to the 2-gallon culture vessels previously described. The plants in the ammonium series were supplied by the continuous renewal method (22) with a solution containing the NH_4 ion as the only source of nitrogen, and those in the nitrate series were supplied with a solution containing the NO_3 ion as the sole source of nitrogen. Each of these solutions was previously known to produce excellent growth of peach trees, and it was supplied within a favorable pH range for normal development, so that the young trees in all the cultures of both series were very vigorous and in excellent condition when the final treatments were established on July 17. On this date the 21 cultures of each series were divided into three groups of 7 cultures each.

During the remainder of the growth period the cultures of the three groups in the ammonium series were supplied with the respective solution used in this series, adjusted to pH 4.0 for the first group, pH 6.0 for the second, and pH 8.0 for the third. The cultures of the three groups of the nitrate series were similarly treated with the respective solution employed in that series, adjusted to corresponding pH values. Each culture was continuously supplied with its

solution at the rate of 1 liter per 24 hours until August 5, when the rate of supply was increased to 2 liters per 24 hours, in order to obviate excessive reaction change of the culture solution as it passed through the sand. On August 15, the rate of supply was raised to 4 liters per culture per 24 hours.

Growth responses and nitrogen absorption. The growth responses of the peach trees in the various treatments were measured by three quantitative

TABLE 2

Total linear growth in centimeters and green and dry weight yields in grams produced per plant in 1931 experiments

DATE	INITIAL pH OF CULTURE SOLUTION	TOTAL LINEAR GROWTH OF STEMS	GREEN WEIGHT			Dry weight			
			Stems	Leaves	Stems plus leaves	Stems	Leaves	Roots	Whole plants
Ammonium series									
August 1	4	187	7.55	16.28	23.83	2.48	3.68	1.38	7.84
	6	257	9.98	22.57	32.55	2.95	5.23	1.47	9.65
	8	197	7.72	17.07	24.79	2.15	8.19	1.58	7.92
August 14	4	205	6.55	16.40	22.95	2.48	4.68	2.15	9.31
	6	326	14.40	34.55	49.45	5.38	8.75	3.20	17.33
	8	271	12.28	27.40	39.68	4.08	6.35	2.45	13.08
August 25	6	491	23.80	46.35	70.15	8.35	11.73	6.75	26.83
	8	391	17.50	36.30	53.80	5.90	8.85	5.50	20.25
Nitrate series									
August 1	4	255	12.82	25.08	37.90	3.77	5.65	2.02	11.44
	6	280	12.92	24.02	36.94	3.87	5.42	2.13	11.42
	8	233	11.07	22.92	33.99	3.43	5.33	1.92	10.68
August 14	4	446	25.78	47.00	72.78	7.95	10.88	4.75	23.58
	6	415	23.73	44.15	67.88	7.83	10.30	5.20	22.83
	8	375	14.73	39.43	59.16	5.93	8.98	4.28	14.19
August 25	4	691	52.08	81.03	133.10	16.23	19.33	8.50	44.06
	6	497	35.98	64.60	100.58	11.50	15.23	8.13	34.86
	8	414	30.40	55.98	86.38	9.98	13.80	7.75	31.53

criteria. The first of these was the measurement made every 5 or 6 days, of the height of the main stem of each plant. These data are supplemented by measurements of the total linear growth of main stems and branches per plant, recorded at each harvest period. The second criterion was that of average green weight per plant per treatment recorded at each harvest period. The third, and perhaps the most important quantitative criterion, was that of average dry weight per plant per treatment determined at each harvest period.

The results of these measurements are presented in table 2, and those of the nitrogen absorption tests are given in table 3, in milligrams of nitrogen absorbed per gram of dry plant material per hour. These data will be considered only very briefly, since the 1931 experiments were preliminary in nature, and since the results agree in general with those obtained in the 1932 experiments, which will be considered in greater detail.

From the data of table 2, it will be observed that the plants in the ammonium series, judged by each of the three criteria, made the best growth in the NH_4-pH-6 treatment, the poorest growth in the NH_4-pH-4 treatment, and growth

TABLE 3

Rates of absorption of ammonium nitrogen and of nitrate nitrogen from nutrient solutions applied at different pH values

DATE OF TESTING	MILLIGRAMS OF NITROGEN ABSORBED PER GRAM OF DRY MATTER PER HOUR					
	Ammonium series			Nitrate series		
	pH 4	pH 6	pH 8	pH 4	pH 6	pH 8
August 1	0.065	0.068	0.085	0.055	0.049	0.031
	0.061	0.075	0.089	0.052	0.046	0.043
Average	0.063	0.072	0.087	0.054	0.048	0.037
August 14	0.092	0.101	0.117	0.061	0.049	0.045
	0.081	0.095	0.132	0.055	0.045	0.039
	0.097	0.097	0.132	0.063	0.050	0.042
	0.090	0.110	0.119	0.058	0.046	0.039
Average	0.090	0.101	0.125	0.059	0.048	0.041
August 25	.....	0.124	0.081	0.065	0.050	0.042
	.....	0.115	0.075	0.055	0.038	0.040
	.....	0.120	0.080	0.053	0.045	0.045
	.....	0.101	0.078	0.059	0.048	0.043
Average	.....	0.115	0.074	0.058	0.045	0.043

intermediate between the two in the NH_4-pH-8 treatment. From a comparison of these plant responses with nitrogen absorption rates in this series, it is apparent that in each of the three tests made, minimum nitrogen absorption rates occur in the NH_4-pH-4 treatments and correspond to minimum growth values, whereas maximum absorption rates occur in the NH_4-pH-8 treatments and correspond to intermediate growth values, maximum growth occurring with the intermediate absorption rates at pH 6.0. These relations hold for the first and second absorption tests, but in the third test, both maximum growth values and maximum absorption rates are shown for the NH_4-pH-6 treatments.

A similar comparison brings out the fact that in the nitrate series maximum, intermediate, and minimum growth values correspond to maximum, intermediate, and minimum nitrogen absorption rates, respectively. These occur in the NO_3-pH-4, NO_3-pH-6, and NO_3-pH-8 treatments, in the order given.

The data indicate clearly that peach trees absorb nitrogen as the cation from a culture solution at relatively high pH much more rapidly than they do from the same solution at relatively low pH; whereas they absorb nitrogen as the anion at a low pH of the culture solution at a much higher rate than they do at a high pH of the culture solution.

The relationships between growth and absorption, as here pointed out, appear to be fairly well defined in spite of the fact that the reaction change of the culture solutions, as they came in contact with the plant roots in passing through the sand cultures, was not adequately controlled, even when the solutions were renewed continuously at the rate of 4 liters per culture per day. It is to be emphasized, therefore, that, although these preliminary experiments yielded results which must be regarded as only approximate, they demonstrated that clearly defined differences in growth and distinct effects resulting from the treatments are to be expected if the reactions of the culture media are maintained within relatively narrow limits around the initial reactions. They have thus served as a guide for the 1932 experiments.

Appearance of plants. A qualitative criterion of considerable importance in judging the plants of these experiments was that of the general appearance. Striking differences occurred in the general appearances of the plants in the different groups of both series. In the ammonium series the leaves of the NH_4-pH-4 plants remained dark green and were duller and smaller than those of normal peach trees. The tissues of these plants were also very hard and rigid. There were, however, no outstanding differences in appearance between the NH_4-pH-6 and the NH_4-pH-8 plants. This is what might have been expected, since throughout the period of the experiments there was little difference in the rates of growth of plants in these treatments.

In the nitrate series, differences in the appearance of the plants in the various treatments were quite obvious but not so striking as the differences in the ammonium series. The NO_3-pH-4 plants had large, dark green leaves, whereas the leaves of the NO_3-pH-8 plants were smaller and lighter green than those of other plants in the nitrate series. The NO_3-pH-6 plants were usually intermediate in appearance between those of the pH-4 and pH-8 treatments, although they resembled closely the NO_3-pH-4 plants.

An obvious feature in a comparison of the plants produced in the two series was the almost complete absence of any iron chlorosis in the ammonium series, as contrasted with the marked tendency of the NO_3-pH-6 plants to become chlorotic—a characteristic which was still more pronounced in the NO_3-pH-8 plants. This is in agreement with the results of others (7, 28, 29). It should be emphasized, however, that the slightest tendency toward chlorosis was

corrected quickly and that no differences in growth response among these plants can be attributed to chlorosis.

Aside from differences in height there were only relatively slight differences in appearance between plants in the pH-6 and pH-8 treatments of the ammonium series and those in the pH-4 and pH-6 treatments of the nitrate series. In figures 1 and 2 of plate 1 are shown typical plants from each series, photographed on August 13, the day before the second absorption test was made.

The response of the root systems of the plants to the reactions used in the two series was even more pronounced than the response of the tops. Thus, in the ammonium series, root growth in the pH-4 treatment was retarded considerably, and by August 14, when the second absorption test and harvest were made, there were many dead rootlets on these plants. Because of this, the pH-4 treatment of the ammonium series was discontinued. The NH_4-pH-6 plants usually produced roots that were somewhat longer than those of the NH_4-pH-8 plants. At the time of the second harvest, some of the roots on the NH_4-pH-6 plants showed some dead rootlets and some discoloration of most of the normally white portions of the root systems. This condition was undoubtedly the result of reaction change from the original pH of the culture solution to a point beyond the range of acid immunity in this treatment. This condition was avoided in later experiments by the use of a larger quantity of culture solution per day with more rapid rates of continuous solution renewal.

In the nitrate series, the best root development took place in the pH-4 treatment. In the pH-6 treatment the upper portions of the root systems produced few rootlets. In the pH-8 treatment of the nitrate series, practically no active root growth was made except at the extreme lower portion of the root systems.

Figures 1 and 2 of plate 2 show typical roots from the pH-6 and pH-8 treatments of the ammonium series, and the pH-4 and pH-6 treatments of the nitrate series, respectively, photographed after the last harvest and absorption test on August 25.

1932 experiments

A group of 40 uniform peach seedling trees, about 25 cm. high, grown as previously described (1), were transplanted into twenty 2-gallon stoneware percolators prepared as in the preliminary experiments of 1931. From August 18 to September 12, all the plants were supplied, by the continuous renewal method, with solution $T_3R_1C_3$, of the modified Tottingham series of Jones and Shive (7), previously tested and known to be an excellent medium for the growth of peach trees. This was done in order to obtain a group of uniform plants of ample size to provide sufficient material for chemical analyses when the plants were harvested at the end of the experimental growth period.

On September 12, the final treatments were established. The 20 cultures of peach trees, now quite uniform in size and general appearance, were divided

into two groups of 10 cultures each. One group was used for the ammonium series, and of these, five cultures were treated with the solution indicated for this series in table 1, adjusted to pH 4, and the other five were treated with the solution adjusted to pH 6. The second group was used for the nitrate series, which was divided likewise into two sets of five cultures each and supplied with the solution indicated for this series in table 1, adjusted to pH values corresponding to those of the solution used in the ammonium series.

Attempts were made to maintain the reactions of the culture solutions in contact with the plant roots within a narrow range about the initial pH values of the respective solutions. Throughout most of the experimental period the reactions of the culture solutions were not altered more than 0.5 of a pH unit while percolating through the sand cultures. Each culture of the two series was supplied continuously with the culture solutions at the rate of 2 liters per day from September 12 to September 24. The rate of supply was then increased to 4 liters per day. On October 17, it became apparent that a still higher rate of supply would be required to maintain the pH of the solutions supplied to some of the cultures within the desired limits. Accordingly the rate of supply was increased to 6 liters per day and then to 8 liters per day which rate was maintained from October 21 to the end of the experimental period.

Because of the large quantities of nutrient solutions required daily after October 17, they were prepared in 90-liter glazed stoneware crocks. The leachings from the cultures were collected and returned to the crocks, where the solutions were readjusted to their initial pH value. These solutions were then used for a second day's supply.

Iron was supplied to the cultures by adding a solution of ferrous sulfate directly to the culture solutions at the rate of 0.2 to 0.4 p.p.m. of iron. Occasionally it was necessary to flush some of the pH-6 cultures of the nitrate series with 400 cc. of a 0.1 per cent solution of $FeSO_4 \cdot 7H_2O$. Chlorosis was always controlled by this treatment.

Growth responses and nitrogen absorption. The peach trees of the 1932 experiments responded more definitely and with much more clearly defined relationships to the cultural treatments applied to them than did the trees in the 1931 experiment. This was undoubtedly due to the much higher rates of continuous solution renewal, which prevented excessive change in the reaction of the culture solution in contact with the plant roots while passing through the sand. However, the general trends and the results, in the main, were quite similar in the two sets of experiments.

Aside from the higher rates of solution renewal, the 1932 experiments were carried out by precisely the same methods as were the preceding ones. Growth responses were measured by the same criteria, and the nitrogen absorption tests were conducted in precisely the same manner in the two sets of experiments.

The nitrogen absorption rates of the plants in the cultures of the two series

were determined only once in connection with the 1932 experiments. These tests were conducted at the end of the experimental period, October 28 and 29, after which the plants were harvested and the green and dry weight yields obtained. The data relating to growth criteria are presented in table 4, and the results of the nitrogen absorption tests are recorded in table 5.

TABLE 4

Total linear growth made during the experimental period, and green and dry yields per plant in the 1932 experiments

	AMMONIUM SERIES		NITRATE SERIES	
	pH 4	pH 6	pH 4	pH 6
	cm.	*cm.*	*cm.*	*cm.*
Total linear growth	132	403	450	306
	gm.	*gm.*	*gm.*	*gm.*
Green weight of plants:				
Stems	12.63	35.02	42.26	27.33
Leaves	24.58	67.02	72.85	50.68
Roots	39.53	65.46	69.88	48.41
Whole plant	76.74	167.50	184.99	126.42
Dry weight of plants:				
Stems	5.03	11.36	14.29	7.92
Leaves	6.62	16.79	18.79	11.09
Roots	6.66	7.56	8.87	6.36
Whole plant	18.31	35.71	41.95	25.37

TABLE 5

Rates of absorption of ammonium and nitrate nitrogen, expressed as milligrams per gram of dry matter per hour by plants in the different treatments of the 1932 experiments

AMMONIUM SERIES		NITRATE SERIES	
pH 4	pH 6	pH 4	pH 6
0.055	0.118	0.080	0.032
0.051	0.112	0.089	0.032
0.055	0.110	0.089	0.039
.....	0.121	0.083	0.032
.....	0.108	0.090	0.031
Average 0.052	0.114	0.086	0.033

The average dry weight yields in grams as given in table 4 and the average absorption rates in milligrams per gram of dry plant material per hour, are brought together for comparison and shown diagrammatically in figure 1. The bars on the left of the zero line represent the nitrate series; those on the right, the ammonium series. Inspection of this diagram shows clearly that in

the ammonium series the high average dry weight yields were produced in the pH-6 treatment and the low yields in the pH-4 treatment, and these are directly related to high and low rates of the absorption of nitrogen as the cation by the peach trees from the pH-6 and from the pH-4 solutions, respectively. In this series, plant measurement values and nitrogen absorption rate values for the trees in the pH-6 treatment are about double the corresponding values for those in the pH-4 treatment.

In the nitrate series, on the other hand, the high yields were produced in the pH-4 treatment and low yields in the pH-6 treatment, and these are directly related to high and low rates of the absorption of nitrogen as the anion by the trees from the pH-4 and the pH-6 solutions, respectively. In this series, plant measurement values of the trees in the pH-4 treatment are nearly double those

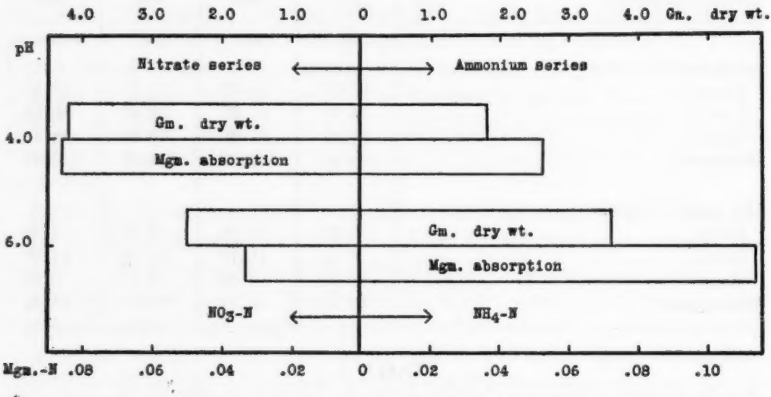

FIG. 1. AVERAGE DRY WEIGHT YIELDS IN GRAMS AND AVERAGE ABSORPTION RATES IN MILLIGRAMS PER GRAM OF DRY PLANT MATERIAL PER HOUR

of the trees in the pH-6 treatment, and the nitrogen absorption rate values for those in the former are nearly three times the corresponding values for those in the latter.

By comparing, now, the yield values and absorption rates for the trees in the ammonium series with those in the nitrate series, as indicated in figure 1, it will be observed that the high average dry weight per tree produced in the pH-6 treatment of the ammonium series is somewhat lower than that produced in the pH-4 treatment in the nitrate series. Similarly, the low dry weight yield produced in the pH-4 treatment of the ammonium series is considerably lower than that produced in the pH-6 treatment of the nitrate series.

On the other hand, cation-nitrogen absorption rates associated with high and low dry weight values in the ammonium series are considerably higher than anion-nitrogen absorption rates associated with high and low dry weight yields in the nitrate series. This means, of course, that the amount of plant material

produced in proportion to the nitrogen absorbed is much higher for the trees in the nitrate series than it is for those in the ammonium series.

As stated in connection with the preceding experiments, measurements were made of the increase in height of the main stem of each tree. From these measurements, recorded every 3 or 4 days, the average daily increment in height of the main stem per plant was calculated for the plants of each treatment in the two series throughout the experimental period. Since these values

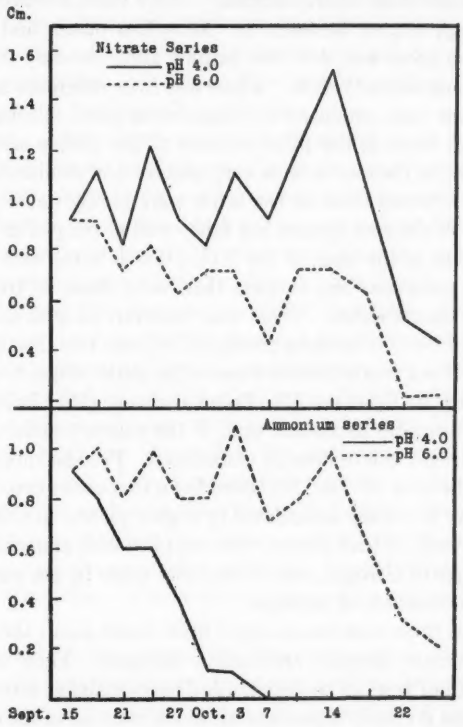

FIG. 2. AVERAGE GROWTH INCREMENTS AS INDICATED BY INCREASE IN HEIGHT OF MAIN STEMS PER DAY

are closely correlated with yields of plant substance, they indicate the trend of the influence of the pH treatments upon the growth of the peach trees over the experimental period from September 16 to October 26. The values of these average daily increments were plotted to form the two sets of graphs of figure 2, the upper set representing the nitrate series and the lower the ammonium series. These graphs clearly show, on the one hand, the complete superiority of the plants in the NO_3-pH-4 treatment over those in the NO_3-pH-6 treatment; and on the other hand, the complete superiority of the plants in the NH_4-pH-6

treatment over those in the NH_4-pH-4 treatment throughout the entire growth period. In fact, as indicated by the lower graph representing the ammonium series, the plants in the NH_4-pH-4 treatment ceased to make any linear growth whatsoever after October 7.

Appearance of plants. In appearance the plants in these experiments were similar to those grown in the corresponding treatments in 1931, except that the characteristic differences resulting from the various treatments were more pronounced and much more clearly defined. There were, however, no significant differences in appearance between the NH_4-pH-6 plants and the NO_3-pH-4 plants except in gross size, and this became apparent only during the last 2 weeks of the experimental period. There was little difference in size or appearance between the roots produced by plants in the pH-4 cultures of the ammonium series and those in the pH-6 cultures of the nitrate series, except that the root systems of the former were very shallow and exhibited comparatively short rootlets; whereas those of the latter were poorly developed throughout the upper half of the root system but fairly well developed in the lower half. The root systems of the trees in the NH_4-pH-6 cultures were more profusely branched into numerous fine rootlets than were those of trees in the pH-4 cultures of the nitrate series. There was, however, no noticeable difference in the gross size of the root systems produced in these two treatments.

In view of the fact that ammonia is one of the initial stages in nitrogen assimilation according to Eckerson (3), Prianischnikow (16), Robinson (19), and others, it is reasonable to assume that, if the proper conditions are supplied, ammonium nitrogen will be directly assimilated. Prianischnikow (18), Pirschle (13), and Tiedjens et al. (28, 29) have shown that under favorable conditions ammonium may be readily assimilated by higher plants, and that in many instances the growth of such plants, when supplied with ammonium compounds as the sole source of nitrogen, may exceed that made by the plants when grown with nitrates as a source of nitrogen.

The results of these experiments leave little doubt about the ability of peach trees to assimilate directly ammonium nitrogen. They show, moreover, that the growth of trees in balanced solutions containing nitrogen only in the ammonium form is closely dependent upon the reaction of the culture medium. Thus it is not enough to supply the culture solution at pH 6 in order to obtain good growth. It is also necessary to supply the solution at this reaction in such quantities as may be required to maintain the pH of the cultures within a favorable range, which, for the peach, appears to be relatively narrow, extending not much above nor much below pH 6.0. This is well illustrated by the superior growth made by the trees in the NH_4-pH-6 treatment during 1932 as compared with that made by the trees in the corresponding treatment of the 1931 experiments. The difference was unquestionably due to the maintenance of a more favorable pH range in the cultures of the 1932 experiments. These results are in agreement with those found by others (12, 13, 28, 29) working with different species.

The very poor growth which occurred in the pH-4 treatment of the ammonium series was not duplicated in any treatment of the nitrate series. All cultures in the latter series appeared to support at least fair growth. This is an indication that the absorption and assimilation of nitrate nitrogen resulting in normal growth of the peach tree are somewhat less dependent upon the reaction of the culture solution than are the absorption and assimilation of ammonium nitrogen. Others (13, 28, 29) have likewise obtained good growth over a relatively wide range of reactions with nitrate treatments. Nevertheless, in the 1932 experiments in which the reaction of the NO_3-pH-6 cultures was maintained within a very narrow range around pH 6 during the growth period, the trees produced only about 60 per cent as much dry plant material as did those grown in the NO_3-pH-4 cultures.

Plant analysis. Immediately after the absorption tests were completed, the plants in both the ammonium and the nitrate series were harvested on October 30 and 31, respectively. The various nitrogenous fractions present in the stems and in the soft, white, fibrous roots were determined. A detailed description of the methods used for the analyses of the nitrogenous constituents of the plants has been presented in a previous publication (2). Unfortunately, however, the method for determining the cyanogenetic nitrogen fraction described in this publication was not available at the time the analyses were made of the plants grown in the 1932 experiments. In these analyses this fraction was represented by the loss of nitrogen during hydrolysis by boiling an aliquot of the soluble nitrogen extract for 2½ hours with 5 per cent sulfuric acid. Subsequent work has shown that this method accounted for only approximately 70 per cent of the cyanogenetic nitrogen present, and that the remaining 30 per cent of this fraction was included with the amide fraction. Corrections have been made for the amounts of cyanogenetic and amide nitrogen determined, and the corrected values for these fractions are included in the tables which follow. The results of the analyses are presented in tables 6 and 7.

In general, most of the nitrogenous fractions, as well as total nitrogen, were present in higher percentages in the stems of plants in the ammonium series than in those of the nitrate series. In proportion to the total organic nitrogen present in the stems, however, there was relatively little difference in the distribution of organic nitrogenous fractions among the plants in any except the NH_4-pH-4 treatment. The stems of plants in that treatment were relatively low in total amino and basic-free amino nitrogen, but they contained approximately twice as high a percentage of basic nitrogen as the stems of plants in any other treatment. The ammonium nitrogen was no higher in the stems of plants grown in the ammonium series than in those grown in the nitrate series. A small but quantitative amount of nitrate nitrogen was found in the stems of plants grown in the NO_3-pH-6 cultures, but no nitrates could be detected, even microchemically, in the stems of trees in any other treatment.

The fibrous roots of plants grown in the ammonium series contained con-

siderably higher proportions of soluble organic nitrogen and of all the soluble organic nitrogenous fractions than the roots of plants in the nitrate series. Roots of the former series also contained higher proportions of ammonium

TABLE 6

Nitrogenous fractions in stems and roots of peach trees in ammonium series, expressed as percentage of green and dry matter, and as percentage of total organic nitrogen

	pH-4 CULTURES			pH-6 CULTURES		
	Green weight basis	Dry weight basis	Total organic N as 100 per cent	Green weight basis	Dry weight basis	Total organic N as 100 per cent
Stems						
Total N	0.684	1.718		0.501	1.524	
Total organic N	0.673	1.692	100.00	0.486	1.479	100.00
Protein N	0.322	0.808	47.77	0.266	0.808	54.61
Soluble organic N	0.351	0.884	52.23	0.220	0.671	45.39
Cyanogenetic N	0.023	0.058	3.42	0.008	0.026	2.93
Amide N	0.073	0.184	10.87	0.060	0.182	12.29
Humin N	0.017	0.041	2.55	0.012	0.038	2.51
Total amino N	0.120	0.302	17.86	0.102	0.311	20.93
Basic-free amino N	0.075	0.188	11.08	0.074	0.226	15.25
Basic N	0.154	0.388	22.91	0.052	0.160	10.77
Ammonium N	0.011	0.027	*1.55	0.015	0.045	*2.91
Nitrate N	.....	.....	.....	.....	.....	.....
Other N	0.009	0.025	1.40	0.014	0.039	1.64
Fibrous roots						
Total N	0.404	3.931		0.287	3.694	
Total organic N	0.393	3.828	100.00	0.272	3.504	100.00
Protein N	0.262	2.547	66.55	0.140	1.802	51.43
Soluble organic N	0.131	1.281	33.45	0.132	1.702	48.57
Cyanogenetic N	0.017	0.169	4.42	0.005	0.070	1.99
Amide N	0.035	0.337	8.80	0.030	0.389	11.11
Humin N	0.008	0.074	1.93	0.009	0.132	3.31
Total amino N	0.049	0.477	12.46	0.056	0.720	20.53
Basic-free amino N	0.031	0.308	8.03	0.034	0.440	12.58
Basic N	0.036	0.350	9.15	0.041	0.529	15.08
Ammonium N	0.011	0.103	*2.62	0.013	0.168	*4.54
Nitrate N	.....	.....	.....	0.002	0.023	*0.63
Other N	0.004	0.043	1.12	0.013	0.142	4.45

* Percentage of total nitrogen.

nitrogen than did those in the nitrate series. The one outstanding difference in composition among the roots grown in the two series is the very high proportion of inorganic nitrogen present as nitrates in the roots of plants grown in the nitrate series.

The assimilation of nitrogen and the distribution of nitrogen fractions. Chemical analyses of the plants in the ammonium and nitrate series show some interesting relationships between growth and composition. The most striking differences in composition occurred in the roots of the two series. This should be expected, however, since the roots are the organs of absorption and, in the case of the peach tree, the chief organs for the initial stages of nitrogen assimilation.

TABLE 7

Nitrogenous fractions in stems and roots of peach trees in nitrate series, expressed as percentage of green and dry matter, and as percentage of total organic nitrogen

	pH-4 CULTURES			pH-6 CULTURES		
	Green weight basis	Dry weight basis	Total organic N as 100 per cent	Green weight basis	Dry weight basis	Total organic N as 100 per cent
Stems						
Total N	0.437	1.267		0.418	1.281	
Total organic N	0.422	1.226	100.00	0.393	1.203	100.00
Protein N	0.219	0.636	51.85	0.218	0.666	55.39
Soluble organic N	0.203	0.590	48.15	0.175	0.537	44.61
Cyanogenetic N	0.013	0.039	3.08	0.014	0.042	3.46
Amide N	0.050	0.146	11.94	0.045	0.138	11.46
Humin N	0.012	0.033	2.65	0.013	0.040	3.36
Total amino N	0.083	0.241	19.72	0.077	0.237	19.66
Basic-free amino N	0.062	0.181	14.79	0.057	0.173	14.41
Basic N	0.047	0.135	11.00	0.047	0.143	11.92
Ammonium N	0.014	0.041	*3.25	0.012	0.037	*2.89
Nitrate N	.....	.....	.....	0.013	0.041	*3.20
Other N	0.019	0.056	4.69	0.001	0.001	.....
Fibrous roots						
Total N	0.283	3.974		0.284	3.675	
Total organic N	0.101	1.420	100.00	0.208	2.692	100.00
Protein N	0.035	0.487	34.24	0.154	1.992	74.04
Soluble organic N	0.066	0.933	65.72	0.054	0.700	25.96
Cyanogenetic N	0.004	0.059	4.15	0.005	0.062	2.31
Amide N	0.016	0.222	15.63	0.014	0.186	6.92
Humin N	0.003	0.041	2.87	0.001	0.016	0.58
Total amino N	0.038	0.536	37.75	0.028	0.357	13.27
Basic-free amino N	0.022	0.303	21.35	0.015	0.197	7.31
Basic N	0.021	0.298	20.95	0.017	0.217	8.08
Ammonium N	0.002	0.034	*0.85	0.003	0.039	*1.06
Nitrate N	0.180	2.520	*63.42	0.073	0.944	*25.67
Other N	.....	0.010	0.77	0.002	0.022	0.76

* Percentage of total nitrogen.

The stems of the trees grown in the ammonium series contained from 19 to 35 per cent more nitrogen than did those of the trees grown in the corresponding cultures of the nitrate series, as shown in tables 6 and 7.

The NH_4-pH-4 plants contained higher proportions of nitrogen in stems and roots than did the NH_4-pH-6 plants. This greater yield in total nitrogen in the stems of the plants of this series can be accounted for entirely by the very high proportion of basic nitrogen found in stems of the NH_4-pH-4 plants as compared with that found in stems of the NH_4-pH-6 plants. In the roots, however, the greater yield of total nitrogen was due to a very high proportion of protein nitrogen.

No significant differences were found in the percentages of nitrogen contained in the stems of plants in the nitrate series.

Tiedjens and Blake (28) found that high yields of dry matter in apple roots were associated with high proportions of total nitrogen. This relation was found to hold for the peach roots grown in the ammonium series, but it did not apply in the case of roots grown in the nitrate series. A direct relation was found in both series, however, when the percentage of total organic nitrogen was compared with the percentage of dry matter in the roots. Total nitrogen in peach roots grown in nitrate cultures can have little relation to assimilation, since the roots may contain very large proportions of inorganic nitrogen as nitrates.

Inorganic nitrogen. The inorganic nitrogen present in the peach roots bears a direct relation to the rate of absorption of nitrogen and to growth. This relation is shown graphically in figure 3, which indicates that, when supplied with an ammonium solution at pH 6, peach roots absorbed nitrogen at a much higher rate and that they contained considerably more ammonium nitrogen than when they were provided with a similar solution adjusted to pH 4. Likewise it is observed in the nitrate series that the roots both absorbed nitrate nitrogen more rapidly and contained a higher percentage of nitrate nitrogen when the culture solution was supplied at pH 4 than when it was supplied at pH 6.

The fact that more than half of the total nitrogen in the absorbing roots of peach trees may exist as nitrates while no nitrates are found in the tops, is a very good indication that amino acid formation in the peach takes place in the roots, just as Thomas (27) found that this process takes place in the roots of the apple tree. Furthermore, since the concentration of ammonium nitrogen was low in the stems of the peach trees in both the ammonium and the nitrate series, and was no higher in the trees of the former than in those of the latter, the indication is that ammonium nitrogen is assimilated likewise mainly in the roots.

It was observed that toward the end of the growing season nitrates usually accumulated in the stems, of peach trees grown in NO_3-pH-6 cultures but were absent from the stems of the NO_3-pH-4 plants. This accumulation of nitrates in the tops, together with the growth responses of these plants, indicates a lack

of ability on the part of the plants to assimilate this form of nitrogen efficiently when grown in cultures with relatively high pH values.

These data are very interesting when studied in comparison with the findings of Emmert (4), who reports as much as 600 p.p.m. of nitrates in the petioles of peach trees grown in the field. It is seen that under the conditions furnished in the NO_3-pH-6 treatment, nitrates accumulated in the stems of the peach trees in concentrations of 130 p.p.m. of nitrogen or 570 p.p.m. of nitrates. The samples with which these determinations were made included a large proportion of wood in which there was no nitrate nitrogen. It is reasonable to assume, therefore, that in the more active and less woody portions of the

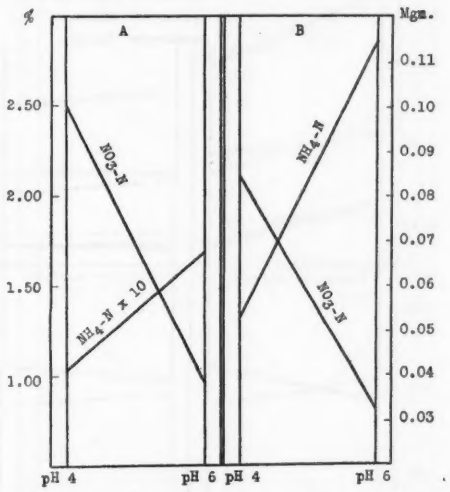

FIG. 3. *A*, INORGANIC NITROGEN IN PEACH ROOTS EXPRESSED IN PER CENT OF DRY MATTER. *B*. RATES OF ABSORPTION OF INORGANIC NITROGEN BY PEACH TREES EXPRESSED IN MILLIGRAMS PER GRAM OF DRY MATTER PER HOUR

stems, the concentrations of nitrates were much higher. One may anticipate, therefore, that nitrates may accumulate in the above ground parts of peach trees grown on heavily limed soil having a high pH value and an abundance of nitrogen in the nitrate form.

Total organic nitrogen. This fraction is composed of protein nitrogen and the various soluble organic nitrogenous constituents. The relation of total organic nitrogen to the other organic nitrogenous fractions in stems and roots of trees in both series is shown graphically in figures 4 and 5. The variations in this fraction among the different treatments have been considered in connection with total nitrogen. It may be observed (fig. 5) however, that in the roots of the NO_3-pH-4 cultures the concentration of total organic nitrogen was very low. Of the total nitrogen in these roots, 63.42 per cent was in

the nitrate form. Nevertheless, this treatment supported the most rapid growth that occurred in any culture. This appears to indicate that nitrogen was transported from the absorbing roots to other parts of the NO_3-pH-4 plants only as rapidly as it was elaborated.

Protein nitrogen. In general, the protein content was relatively high in those plants which grew slowly and produced low yields of dry plant substance. The proportions of protein nitrogen present in the stems and roots of trees grown in the several pH treatments are shown diagrammatically in figures

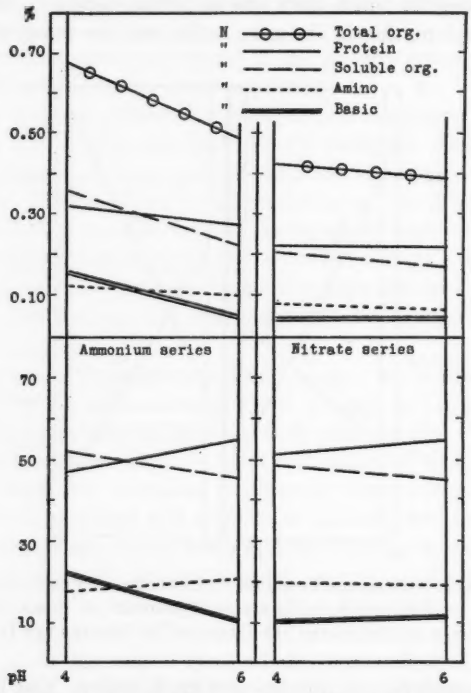

FIG. 4. NITROGENOUS FRACTIONS IN STEMS OF PEACH TREES EXPRESSED IN PER CENT GREEN MATTER (ABOVE), AND IN PER CENT TOTAL ORGANIC NITROGEN (BELOW)

4 and 5. These indicate clearly that the low yields of dry plant material made by the NH_4-pH-4 plants were associated with a high proportion of protein nitrogen in their stems and roots, whereas the high yields made by the NH_4-pH-6 plants were associated with a relatively low proportion of protein nitrogen in both stems and roots. This is in agreement with the work of Sessions and Shive (21). By a similar manner of comparison, the trees in the NO_3-pH-6 cultures, which showed lower yields of dry matter than those in the NO_3-pH-4 cultures, contained relatively higher proportions of protein nitrogen than were found in the latter plants.

Soluble organic nitrogen. The soluble organic nitrogen found in the peach trees grown in the 1932 experiment was composed mainly of amide, amino, and basic nitrogen. The analyses show that, with the exception of the stems of the NH_4-pH-4 plants a relatively high proportion of soluble organic nitrogen was always associated with high yields of dry plant substance in each of the two series.

Cyanogenetic nitrogen. There appears to be no obvious relation between the content of cyanogenetic nitrogen and the growth responses of the trees

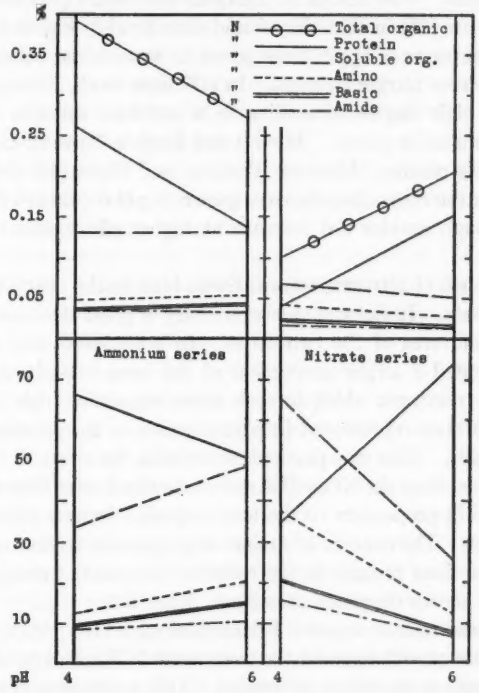

FIG. 5. NITROGENOUS FRACTIONS IN PEACH ROOTS EXPRESSED IN PER CENT GREEN MATTER (ABOVE), AND IN PER CENT TOTAL ORGANIC NITROGEN (BELOW)

or yield values in these experiments. It is probable that this form of nitrogen may serve as a quickly available reserve nitrogenous fraction, although there was no clearly defined evidence to indicate that it served such a function in these experiments. Robinson (20) has shown that this fraction is subject to wide seasonal variations, and that, at times, it may represent a major nitrogenous fraction.

Amide nitrogen. Suzuki (25, 26) believed that amides serve as intermediate products in the synthesis of proteins from nitrates. Later Prianischnikow (14)

claimed that amides are an intermediate form of nitrogen both in the synthesis of protein from ammonia and in the hydrolysis of protein to ammonia. Prianischnikow (15) also found that amide nitrogen was formed more rapidly in *Vicia sativa* and in *Zea mays* seedlings when supplied with ammonium salts together with calcium carbonate than when calcium nitrate served as the source of nitrogen in the growth medium. Nightingale (10, 11) found that amides increase at the expense of proteins when plants are kept in darkness, showing that amides are certainly intermediate nitrogenous compounds formed during proteolysis. The results of Tiedjens and Blake (28) confirmed those of Prianischnikow (15) in that they found considerably higher concentrations of amides in the roots of apple trees grown in ammonium cultures than they found in roots from nitrate cultures. In still later work, Prianischnikow (16) found that a high degree of acidity in a nutrient solution hindered the formation of amides in plants. Mevius and Engle's (8) work did not support Prianischnikow's results. Likewise Tiedjens and Blake (28) did not find any more amides in the roots of apple trees grown in pH-6 cultures than in pH-4.5 cultures, although amides did increase at higher pH values of the culture medium.

The amide form of nitrogen was relatively high in the peach trees grown in these experiments. It did not, however, vary a great deal among the trees grown in the cultures of the two series. In both stems and roots, amides always represented a larger proportion of the total organic nitrogen in the plants of that treatment which in each series supported high yields of plant substance than they represented in plants grown in the treatment that supported low yields. This was particularly true in the roots of trees grown in the nitrate series, since the NO_3-pH-4 roots contained more than twice as much amide nitrogen in proportion to the total organic nitrogen present as did the NO_3-pH-6 roots. The content of amides in proportion to the content of other nitrogenous fractions present in the roots of the peach trees grown in these experiments is shown diagrammatically in figure 5.

Although these results support Prianischnikow's (16) views, it is doubtful whether the poor growth made by the trees in the NH_3-pH-4 treatment resulted from an inadequate synthesis of amides. This expression of doubt is warranted since the NH_4-pH-$_4$ plants actually contained a much higher percentage of amide nitrogen, although it was less in proportion to the other nitrogenous fractions than was found in the NO_3-pH-4 plants.

Humin nitrogen. According to Holme and Gortner (6), the acid-insoluble humin fraction is probably formed from tryptophane. It therefore represents one of the more complex soluble organic nitrogenous constituents of the peach. Tiedjens and Blake (28) found that humin nitrogen in the roots of apple trees was more closely associated with the growth of the trees than were the amino and amide fractions. Humin nitrogen usually represented the smallest organic nitrogenous fraction found in either the stems or the roots of the trees produced in the two series of these experiments. Nevertheless, the highest percentage

value of this fraction was directly associated with the highest yield values of plant substance in each of the two series.

Amino nitrogen. Although amino acids represent assimilated nitrogen in plants, they do not represent the final stages of assimilation. They probably serve as the chief forms of transportable, elaborated nitrogen in higher plants. In the peach trees of these experiments, as Thomas (27) found in the apple, inorganic nitrogen is probably elaborated largely to the amino form in the roots, and is then translocated to other parts of the plant. Considerable evidence is available in support of this suggestion.

It will be observed in tables 6 and 7 that the total amino nitrogen, expressed on a dry weight basis, was always highest in the stems and roots of trees in that treatment which, in each of the two series, supported the best growth. Thus a direct relation is shown between the content of amino nitrogen in the plants and their growth responses.

The same relation also holds for basic-free amino nitrogen and growth. The significance of the variations between total and basic-free amino nitrogen in the various treatments, however, is not clear.

These experiments support the findings of Tiedjens et al. (28, 29) that plants grown in media with ammonium as the source of nitrogen contain a higher percentage of amino nitrogen than do plants grown correspondingly in media with nitrate as the source of nitrogen.

Basic nitrogen. As determined in these analyses, basic nitrogen supposedly includes the basic amino acids and some peptide nitrogen. The fraction is therefore composed of somewhat more complex forms than the simple amino acids. With this in mind, it is interesting to study this fraction in the roots and stems of plants grown in the different treatments.

Invariably the stems of plants which made the best growth in each of the two series contained the lowest percentage of basic nitrogen, whereas the roots of the same plants contained the highest percentage of basic nitrogen. Thus the NH_4-pH-6 plants contained a lower percentage of basic nitrogen in their stems but a higher percentage in their roots than was found in the NH_4-pH-4 plants. Similarly, the NO_3-pH-4 plants contained a lower percentage of basic nitrogen in the stems but a higher percentage in the roots than did the NO_3-pH-6 plants.

The condition that existed in stems of the NH_4-pH-4 plants is particularly interesting. It was mentioned previously that these plants were relatively low in protein nitrogen for slowly growing plants. Moreover, this condition was associated with a very high concentration of basic nitrogen. Also, the amino nitrogen content of these stems was probably no lower in comparison with that found in the NH_4-pH-6 stems than the growth responses of the plants would indicate. Since the basic fraction may include considerable peptide nitrogen, it would seem to indicate that protein synthesis in these stems was probably being retarded in the peptide stage.

SUMMARY

Peach trees were grown in sand cultures in two series of treatments, in one of which the cultures received nitrogen only in the ammonium form; in the other, they received nitrogen only in the nitrate form. The nutrient solutions in both series of treatments were applied at pH 4, 6, and 8. Absorption tests were conducted to determine the rates, in milligrams per gram of dry plant material per hour, at which nitrogen was absorbed by plants in the various treatments. Quantitative analyses were made to determine the relative distribution of various nitrogenous fractions in the plants grown in the treatments. The more important results may be summarized briefly as follows:

Peach trees made much better growth in sand cultures supplied continuously with a culture solution at pH 6 than at pH 4 or pH 8, with NH_4 as the sole source of nitrogen.

Peach trees made much better growth in sand cultures supplied continuously with a culture solution at pH 4 than at pH 6 or pH 8 with NO_3 as the sole source of nitrogen.

The trees grew about equally well in sand cultures at pH 6 with ammonium as the source of nitrogen and at pH 4 with nitrate as the source of nitrogen.

Ammonium nitrogen was absorbed at higher rates by trees supplied with solutions adjusted to pH 6 than by those supplied with solutions adjusted to pH 4.

Nitrate nitrogen was always absorbed at higher rates by trees supplied with solutions adjusted to pH 4 than by those supplied with solutions adjusted to pH 6 or 8.

At favorable pH values of the culture solutions, peach trees absorbed nitrogen at relatively higher rates when it was supplied in the ammonium form than when it was supplied in the nitrate form.

The initial stages of ammonium assimilation by the peach take place in the roots.

Nitrate reduction by peach trees takes place almost entirely in the roots.

Good growth and high yields of plant substance were usually associated with relatively low percentages of protein nitrogen, both in stems and in roots.

Good growth and high yields of plant substance were associated with relatively high percentages of amide, amino, and humin nitrogenous fractions.

Invariably the stems of trees that made the best growth and produced highest yields of plant substance were relatively low in basic nitrogen, while the roots of the same plants were relatively high in this fraction.

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PLATE 1

FIG. 1. Representative trees grown in the ammonium series. Left, NH_4-pH-4 culture; right, NH_4-pH-6 culture.

FIG. 2. Representative trees grown in the nitrate series. Left, NO_3-pH-4 culture; right, NO_3-pH-6 culture.

PLATE 1

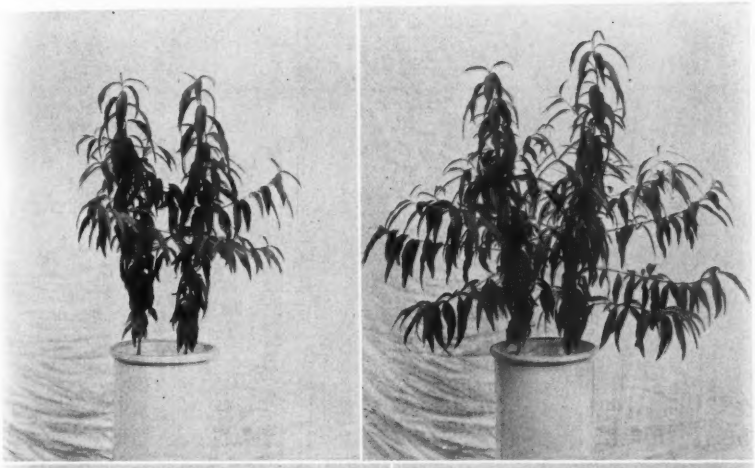

FIG. 1

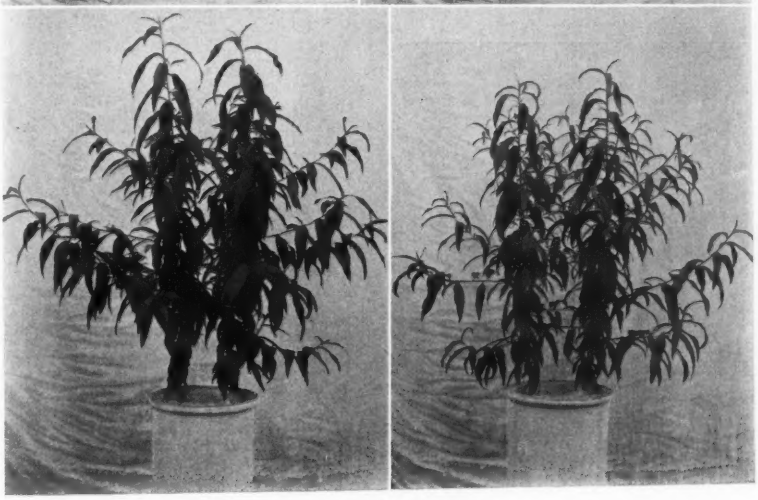

FIG. 2

PLATE 2

FIG. 1. Roots of representative trees grown in the ammonium series. Left, NH_4-pH-6 culture; right, NH_4-pH-8 culture.

FIG. 2. Roots of representative trees grown in the nitrate series. Left, NO_3-pH-4 culture; right, NO_3-pH-6 culture.

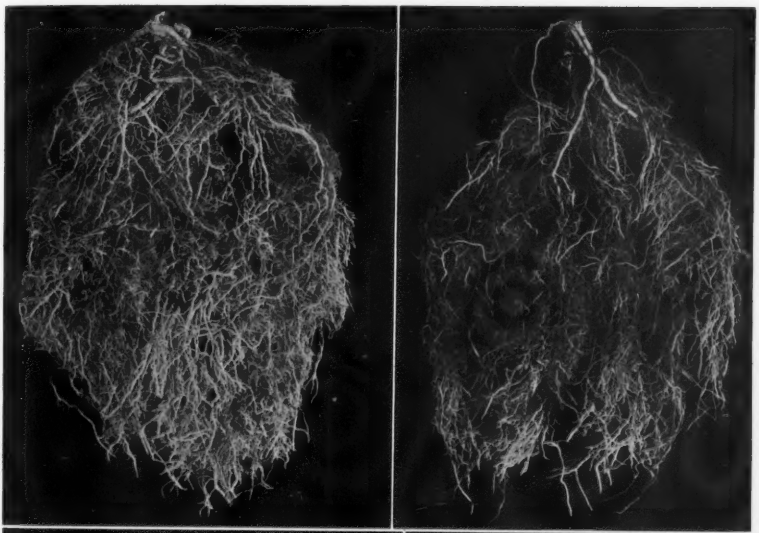

FIG. 1

FIG. 2

PHYSIOLOGICAL STUDIES ON RHIZOBIUM: I. THE EFFECT OF NITROGEN SOURCE ON OXYGEN CONSUMPTION BY RHIZOBIUM LEGUMINOSARUM FRANK[1]

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The discovery of the symbiotic relationship between leguminous plants and the root-nodule bacteria quite naturally led to the assumption that as the bacteria are the active agents in the fixation of nitrogen by inoculated legumes they should be able to fix nitrogen non-symbiotically when cultured in a suitable medium. The early work of Beijerinck (2) indicated that this was actually the case, although he did not find appreciable amounts of nitrogen fixed in his cultures. Later workers were more definite in concluding that the bacteria are capable of fixing nitrogen in the absence of the legume plant.

The work of a number of investigators in recent years, however, has thrown considerable doubt upon this assumption. The studies of Hopkins (10), Pohlman (13), Allison (1), Löhnis (11), and Wilson, Hopkins, and Fred (19) have been outstanding in this connection. All of these investigators came to the conclusion that under the conditions of their experiments, which were extremely variable, there was not a significant amount of nitrogen fixed by the organisms when grown alone. It has even been shown in Burk's (6) respiration studies with Rhizobium, that the organisms would not grow in a medium devoid of nitrogen. When ammonium or nitrate nitrogen was added growth occurred, as indicated by the consumption of oxygen by the organisms. Other investigators also have shown the desirability of adding small amounts of nitrogen to media in which the organisms are to be grown. Water extracts of leguminous plants, yeast extract, asparagin, nitrate, ammonium, and other forms of nitrogen have been used for this purpose.

Pohlman (13), although he obtained negative results, suggests that if proper environmental conditions are supplied, the root-nodule bacteria may be able to fix nitrogen when grown alone. After reviewing the literature on this subject Fred, Baldwin, and McCoy (8) concluded that "It is at least possible that sometime a new medium or new conditions of experiment may be found which will allow fixation." Although it seems that practically every known combination of conditions has been tried by the various investigators of this problem, there are still possibilities. As an example of what may be done, the recent work of Burk (7) may be cited. Until very recently it was thought absolutely

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necessary to provide strictly aerobic conditions for the growth of Azotobacter, but by applying the theory of biological oxidation and reduction, as developed by Weiland and described by Buchanan and Fulmer (5), Burk has succeeded in growing Azotobacter under strictly anaerobic conditions, simply by furnishing the organisms with a suitable hydrogen acceptor in place of the free oxygen available under aerobic conditions. Although this work has no direct bearing upon the problem of nitrogen fixation by Rhizobium, it does, along with the recent work of Winogradsky (20) and of Blom (3), suggest that there is a possibility of providing a certain set of conditions under which the organisms would use free nitrogen as the hydrogen acceptor in the oxidation of carbohydrates for growth energy. What these conditions might be, remains to be determined, and investigational work along this line will certainly be worthwhile.

The whole problem of the nitrogen nutrition and metabolism of these bacteria is involved, and studies along these lines would undoubtedly contribute much toward an understanding of the process of nitrogen fixation as it is effected by the microörganisms. The results of such work would not only be of considerable scientific interest but they would have an influence upon the practice of soil and seed inoculation. Studies on the nitrogen metabolism of the bacteria of the genus Rhizobium have been outlined, therefore, and the present paper is a report of one phase of this work. It deals with the effect of various sources of nitrogen on the consumption of oxygen by *Rhizobium leguminosarum* Frank, the type species of the genus.

EXPERIMENTAL

Various procedures suggest themselves for studying the effects of free nitrogen and of various kinds of combined nitrogen on the metabolism of Rhizobium. The organisms may be grown on the surface of agar containing various nitrogen compounds and observations made of growth by the rapidity and extent of colony formation. This may be considered a qualitative test. Liquid cultures in the proper media may be used and comparative amounts of growth determined by turbidity methods. This is also a qualitative method. To obtain results of a quantitative nature, the numbers of organisms in liquid cultures at various intervals might be determined and the generation time, which is a good measure of rate of growth, calculated. This method was used in previous studies on Rhizobium by Walker, Anderson, and Brown (16).

In order to simplify the procedure, however, and at the same time obtain a more delicate measure of the effects of nitrogen on the metabolism of Rhizobium, new methods were sought. After considerable investigation respiration methods, used rather widely by the physiologists and to some extent by bacteriologists, were adopted. The particular apparatus and technic employed were developed by Warburg (17) and will be described briefly.

Method

Otto Warburg and his associates in Germany have been engaged for some years in a study of the metabolism of cells, particularly cells of tumors and cancer tissue, by the respiration method using specially devised respiration chambers attached to micromanometers. By this means it was possible to measure minute amounts of oxygen consumed and carbon dioxide produced by the cells. Essentially the same technic has been used extensively by Crozier at Harvard and by Burk at the Bureau of Chemistry and Soils.

The principal apparatus employed is a specially constructed vessel in which the organisms are grown, and to which a "U"-shaped manometer of capillary bore is attached by means of a ground glass joint. These are shown in figures 1 and 2 of plate 1.

Inside the culture vessel there is sealed a glass cup which holds an alkaline solution for absorbing the carbon dioxide produced by the organisms. In these experiments 0.3 cc. of 2 *N* KOH was employed for this purpose. The culture of organisms (2 cc.) is placed in the flask. As the organisms respire they consume oxygen from the atmosphere within the flask, inasmuch as they are aerobic. If the stopcock at the top of the manometer arm is closed to prevent contact between the outside atmosphere and the atmosphere within the flask, there will be a negative pressure developed in the flask as the organisms consume oxygen. This change in pressure is indicated by the level of the confining fluid within the manometer. Brodie's solution, because of its low specific gravity, is well adapted for use as this confining liquid. It has a standard pressure of 10,000 mm. compared with 760 mm. for mercury. It flows through the capillary of the manometer with ease and does not adhere to the sides of the tube. It may be prepared by dissolving 23 gm. of sodium chloride and 5 gm. of sodium choleate in 500 cc. of water and adding one drop of a concentrated alcoholic solution of thymol as a preservative. As the solution is colorless, it has been found desirable to add a little dye, such as neutral red, to facilitate the reading of the manometer.

The amount of oxygen consumed by the organisms may be calculated from the changes in pressure by use of the following equation developed by Warburg (17):

$$X = h\left(\frac{V_g - \frac{273}{T} + V_f \alpha_{O_2}}{P_o}\right)$$

where X = oxygen consumed in cubic millimeters, h = change in pressure at constant volume in millimeters of manometer fluid (Brodie's fluid), V_g = volume of gas space in cubic millimeters between the surface of liquid medium and the meniscus of the confining fluid in the manometer, V_f = volume of culture medium in cubic millimeters, α_{O_2} = the solubility coefficient (Bunsen's coefficient) of oxygen in the medium, P_O = standard pressure in millimeters of manom-

eter fluid (10,000 mm. Brodie = 760 mm. Hg). For any given vessel at constant temperature and with constant V_f the term within the bracket is a constant. Thus the change in height of the manometer fluid, h, multiplied by the vessel constant gives the cubic millimeters of oxygen consumed.

In the oxygen consumption experiments reported here a series of seven manometers were employed. The culture vessels were kept in a large water bath with the temperature thermostatically controlled at 28° ± 0.01°C. Figure 3 of plate 1 shows the manometers and the water bath as used in the experiments.

In all experiments one manometer and one vessel were used as a thermobarometer to serve as a control against changes in barometric pressure and minor changes in temperature. This was found to be very desirable since small fluctuations in barometric pressure may make an appreciable change in the level of the Brodie's solution and yet the change in barometric pressure would be so small that it would be imperceptible by the ordinary mercury barometer. Furthermore, the corrections of gas volumes for changes in atmospheric pressure were greatly facilitated by the use of one manometer as a thermobarometer.

Preparation of cultures

A basic nitrogen-free medium of the following composition was prepared and used in all the experiments:

Glucose	10.0 gm.
K_2HPO_4	0.5 gm.
$MgSO_4$	0.2 gm.
NaCl	0.1 gm.
$CaCl_2$	0.1 gm.
Distilled water	1,000.0 cc.

In the various experiments different materials were added to this basic medium as sources of nitrogen. Ammonium chloride, sodium nitrate, urea, d-l-alanine and bacto-yeast-extract were employed as sources of nitrogen, and the organisms were grown in various concentrations of each of these materials. The concentrations of nitrogen in the culture media of different tests were as follows: none, 0.72, 7.2, 36.0, 72.0, and 144.0 p.p.m. of solution.

The organisms used in the experiments were rapidly growing cultures of the root-nodule bacteria of garden peas, *Rhizobium leguminosarum*. The inoculum was prepared by first growing the organisms on a large slant of yeast-mannitol agar containing 0.5 gm. of bacto-yeast-extract per liter. To prevent transfer of appreciable quantities of nitrogen from the yeast-mannitol slant the organisms were washed off with a small amount of sterile distilled water. One cubic centimeter of the resulting suspension of organisms was placed into the manometer vessel to which had been added 1 cc. of the sterile medium (double strength) in which the organisms were to be grown. The 1 cc. of inoculum for

each manometer vessel was taken from the same suspension of organisms for any one experiment to insure uniform inoculation of all vessels. After the culture had come to the temperature of the water bath and sufficient time had elapsed to permit complete absorption of the carbon dioxide of the atmosphere within the manometer vessel, the manometer stopcock was closed and the experiment started. In each case the tests were conducted over a period of 30 hours; changes in the level of the manometer fluid being observed at intervals of 1, 2, or 3 hours.

RESULTS

In the first test a culture of *Rhizobium leguminosarum* was grown in the basic medium described earlier, containing various concentrations of nitrogen,

TABLE 1

Oxygen consumption by Rhizobium leguminosarum *in media containing yeast extract*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as yeast extract in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
1	6.4	7.7	10.6	10.6	11.9	11.1
3	16.6	18.5	29.0	30.4	36.4	35.3
5	27.9	32.5	52.6	58.3	68.8	67.5
7	38.4	45.9	76.2	93.7	109.4	113.0
9	48.5	59.3	100.2	138.5	164.0	173.5
11	57.4	71.2	120.0	186.6	223.5	245.9
12	61.4	76.8	131.3	213.8	258.2	288.7
14	72.3	87.9	149.0	261.9	317.0	364.2
16	79.7	97.6	167.1	313.5	380.4	441.7
19	96.4	114.7	191.9	387.3	475.9	556.6
22	108.4	128.4	213.0	457.3	568.4	668.2
25	120.1	143.3	234.2	518.8	663.0	779.3
28	131.2	157.7	256.8	578.2	757.4	890.1
30	138.6	166.2	270.9	615.0	818.9	961.1

added in the form of bacto-yeast-extract, and in the same basic medium without nitrogen. The total amounts of oxygen consumed by the organisms in the different media are shown in table 1. From the data in the table the rates of oxygen consumption were calculated. These are shown graphically in figure 1.

The results show that the total amount of oxygen consumed varied with the amount of nitrogen in the medium. It is evident, however, that respiration was being carried on by the organism in the no-nitrogen medium, but the amount of oxygen consumed was not large, when compared with the amount consumed in media containing appreciable amounts of nitrogen. Furthermore, the oxygen consumption decreased at nearly a uniform rate throughout the

experiment. This would indicate that although the cells were alive and respiring, they were not multiplying, and furthermore, that their respiration rate was gradually decreasing. In other words, they might be looked upon as resting cells, as the term is used by Quastel and his associates (14).

In the various media containing nitrogen the organisms reacted somewhat differently. The nitrogen stimulated growth and multiplication of the cells in the cultures, resulting in an increased rate of oxygen consumption, which in turn led to the consumption of larger amounts of oxygen.

In the medium with only 0.72 p.p.m. of nitrogen the stimulation was not great but it was definite between the fourth and eighth hours. After the eighth hour the rate decreased for a few hours, after which it was fairly uniform, the rate curve being almost parallel with the rate curve of the no-nitrogen medium during the same period. This indicates that during the early part of

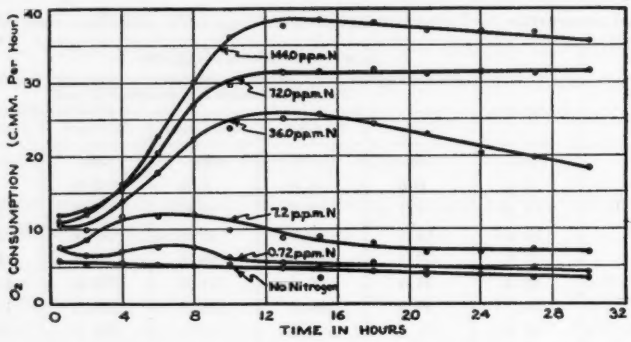

FIG. 1. THE RATE OF OXYGEN CONSUMPTION BY *Rhizobium leguminosarum* IN YEAST EXTRACT MEDIA

the experiment growth was stimulated by the nitrogen. The amount of nitrogen available, however, was extremely small and was soon used by the organisms. After it was all used the rate of oxygen consumed decreased and then continued at a low uniform rate. It appears that after utilization of the available nitrogen the cells continued to respire but did not grow and were in a state similar to the resting cells in the no-nitrogen medium.

Where larger amounts of nitrogen were available practically the same influence on the rate of oxygen consumption occurred, except it was magnified almost in proportion to the increase in nitrogen content in the medium. The increase in rate of oxygen consumption was very rapid and continued for a period of about 12 hours. During this period the organisms were undoubtedly growing rapidly and it is quite probable that they were in their logarithmic phase of growth. After that time the rate in most cases was fairly uniform, except for a slight decrease in the medium with 36.0 p.p.m. of nitrogen, indicating that the organisms were probably in a resting condition. These findings

TABLE 2

Oxygen consumption by Rhizobium leguminosarum *in media containing ammonium chloride*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as ammonium chloride in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	10.5	11.6	7.4	7.8	8.8	10.2
4	21.2	23.8	16.2	16.1	17.0	17.5
6	32.9	33.5	23.7	22.3	23.4	22.9
8	42.7	43.6	31.1	30.5	30.4	28.7
10	53.8	55.5	39.6	39.7	40.2	36.2
12	62.4	65.9	47.1	46.8	47.9	43.7
15	76.5	82.8	61.4	58.7	61.0	56.7
18	91.7	99.3	75.3	74.3	75.7	69.6
20	100.2	110.4	82.9	83.1	84.4	76.7
22	109.5	125.3	92.1	92.6	93.9	84.4
24	118.1	141.7	103.0	105.3	104.4	92.6
26	127.4	160.3	117.1	124.7	115.6	102.4
28	135.8	179.5	132.7	139.1	130.0	111.2
30	144.4	198.4	147.6	158.9	144.0	118.7

TABLE 3

Oxygen consumption by Rhizobium leguminosarum *in media containing sodium nitrate*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as sodium nitrate in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	17.0	16.8	14.6	14.6	15.8	15.6
4	27.1	27.2	25.2	23.8	25.2	23.1
6	37.7	39.1	35.1	33.4	34.8	30.3
8	47.3	48.2	44.1	42.3	43.5	37.0
10	56.2	58.0	54.4	49.8	51.7	45.2
12	66.9	68.6	66.4	59.6	61.6	56.5
14	77.0	79.3	77.7	69.1	71.0	67.4
16	86.4	90.2	91.0	78.7	81.5	79.4
18	94.7	101.7	100.8	86.8	89.9	89.9
20	103.2	114.6	113.1	94.9	99.7	100.6
22	.....	129.9	126.9	107.3	111.4	113.6
24	125.5	145.7	140.7	117.9	123.1	126.2
26	134.3	161.5	152.9	128.4	135.1	139.2
28	141.5	175.5	165.1	139.4	147.1	152.2
30	149.9	191.3	179.9	151.8	159.9	166.7

TABLE 4

Oxygen consumption by Rhizobium leguminosarum *in media containing urea*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as urea in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	10.2	8.9	7.8	7.4	6.3	7.7
4	16.6	14.6	14.6	13.5	12.6	13.1
6	22.8	20.1	23.6	18.7	18.2	18.5
8	29.0	25.3	27.6	23.2	23.4	23.5
10	35.7	32.3	32.5	28.3	28.1	28.5
12	42.2	39.4	37.7	34.7	34.6	35.2
15	53.3	49.1	48.2	42.9	42.7	42.7
18	60.5	59.2	57.7	52.8	51.4	50.2
21	70.9	70.2	68.6	63.3	61.4	58.6
23	76.3	76.3	74.3	68.4	65.3	61.7
25	82.1	82.5	80.7	75.3	71.4	65.4
28	90.7	92.2	90.6	85.2	79.5	70.8
30	96.3	98.4	94.4	91.3	84.4	73.8

TABLE 5

Oxygen consumption by Rhizobium leguminosarum *in media containing alanine*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as alanine in media				
		0.72 p.p m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
0	0.0	0.0	0.0	0.0	0.0	0.0
2	13.3	7.7	14.4	10.7	14.0	21.7
4	22.4	16.6	20.1	15.9	20.3	33.1
6	31.0	23.3	23.6*	24.7	31.7	48.7
8	39.6	30.7	30.0	31.8	39.4	60.9
11	50.7	42.6	40.3	43.1	52.7	77.2
14	61.8	53.8	53.6	52.6	62.1	90.1
17	72.3	65.7	71.7	62.9	73.4	103.7
20	81.6	77.6	80.2	72.8	84.2	117.6
23	92.1	91.0	97.9	82.0	94.0	130.2
26	103.5	106.3	127.9	93.7	106.6	145.7
28	111.3	116.3	144.9	101.8	114.3	156.0
30	118.1	123.0	156.2	106.0	119.9	164.8

are in accord with the earlier conclusions of Walker, Anderson, and Brown (16) concerning the period of the logarithmic phase of growth for Rhizobium.

Tables 2, 3, 4, and 5 show the results obtained in similar oxygen consumption experiments where other forms of nitrogen, ammonium chloride, sodium nitrate, urea, and alanine, were used in place of the yeast extract. The rates

of oxygen consumption were calculated from these data and some representative results are shown in figure 2.

These data indicate that *Rhizobium leguminosarum* reacted entirely differently when nitrogen was supplied in forms other than yeast extract. In general, the rate of respiration, and therefore, the total amount of oxygen con-

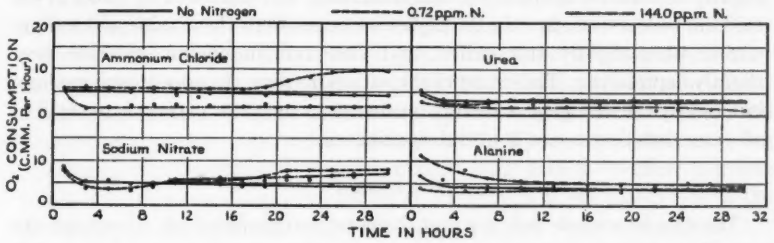

FIG. 2. THE RATE OF OXYGEN CONSUMPTION BY *Rhizobium leguminosarum* IN MEDIA CONTAINING AMMONIUM CHLORIDE, SODIUM NITRATE, UREA, AND ALANINE

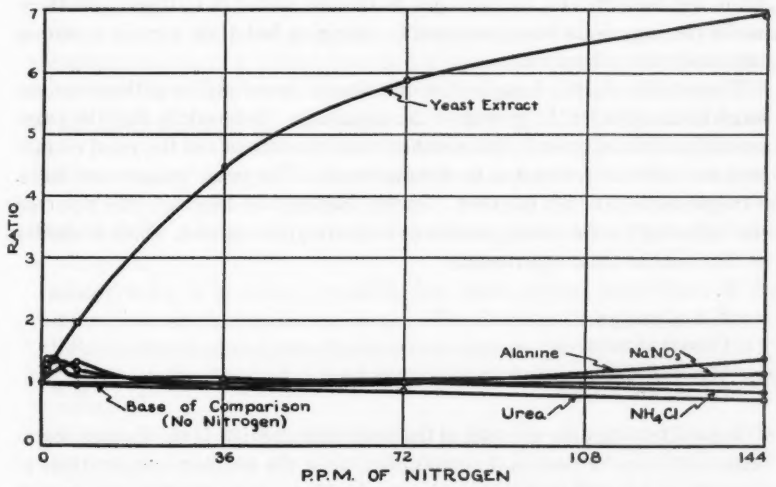

FIG. 3. RATIO OF THE AMOUNT OF OXYGEN CONSUMED IN 30 HOURS IN MEDIA CONTAINING VARIOUS NITROGEN SOURCES TO THAT CONSUMED IN A NITROGEN-FREE MEDIUM

sumed, was very nearly the same in all of the media, irrespective of the source or concentration of nitrogen used. In no case was there any large stimulation of oxygen consumption in the media containing nitrogen. The larger amounts of urea and ammonium chloride, however, depressed oxygen consumption slightly, but no depression of oxygen consumption occurred with the sodium nitrate or the alanine.

Figure 3 shows graphically the ratio of the total amounts of oxygen con-

sumed in the different concentrations of the various nitrogenous materials compared with that consumed in the no-nitrogen medium at the end of 30 hours. These curves show definitely the difference in the response of the organisms to the various materials. The yeast extract was decidedly stimulative in all concentrations, whereas the other materials appeared to be only slightly stimulative in the lower concentrations, and of little or no effect in the medium concentrations. In the higher concentrations the alanine and sodium nitrate were slightly stimulative, and urea and ammonium chloride were slightly depressive. The extent of the variations from the no-nitrogen medium induced by the nitrogenous materials other than the yeast extract is so small in all cases that they are of doubtful significance.

DISCUSSION

The data as a whole indicate that the yeast extract definitely stimulated the growth of *Rhizobium leguminosarum*, whereas the other nitrogenous materials used did not appreciably stimulate growth. The organisms in the media containing ammonium chloride, sodium nitrate, urea, or alanine reacted as they did in the medium with free nitrogen as the sole source of nitrogen. In these media the organisms were, presumably, respiring but they were in a resting state and were not growing.

The results naturally bring up the question of the suitability of these various forms of nitrogen for the growth of the organisms. It is certain that the yeast extract stimulated growth, but whether it was the nitrogen of the yeast extract that was effective remains to be demonstrated. The yeast extract used has a nitrogen content of 7.6 per cent. Sadler, Eagles, and Pendray (15) reported the following for the nitrogen content of bacto-yeast-extract, which is similar to that used in these experiments:

	per cent
Protein nitrogen	0.0
Proteose nitrogen	0.0
Peptone nitrogen	24.2
Sub-peptone nitrogen	75.8

It is evident that the nitrogen of the yeast extract exists in the simpler degradation products of protein decomposition, or of the simplest combinations of the amino acids, and presumably nitrogen in this form is suitable for the growth of Rhizobium. Then there is also the possibility that in the presence of the other constituents of the yeast extract the organisms were better able to utilize the free nitrogen of the atmosphere.

It is entirely possible that there were certain constituents in the yeast extract other than the nitrogen that caused the stimulation in oxygen consumption. It is not known what these may have been, but undoubtedly certain readily available carbonaceous materials were present. It is a question, though, whether such materials could have been much more effective in stimulating oxygen consumption than the glucose, which was present in all the media.

Glucose ordinarily offers a readily available source of energy for the organisms and is usually considered entirely satisfactory for this purpose. The use of mannitol in yeast extract medium has been recommended by Fred and Waksman (9), and is used extensively by some investigators working with Rhizobium. In comparative tests, however, respiration experiments with mannitol in the medium in place of glucose have shown no superiority for the mannitol in stimulating growth, as determined by oxygen consumption.

There is also the possibility that the yeast extract contained some accessory food constituent such as vitamins or auximones. Bottomley (4) and Mockeridge (12) concluded that certain microörganisms synthesize accessory food constituents, which they called "auximones." When materials containing auximones were supplied to growing plants along with a complete nutrient solution there was a direct stimulation of the growth of the plants due to the auximones. The beneficial effects of bacterized peat were attributed to the auximones produced by the bacteria. If it is possible for microörganisms, and particularly yeasts in this case, to produce such constituents, then the stimulation of the growth of Rhizobium may have been due to the auximones of the yeast extract.

Then too, if yeast cells are capable of synthesizing vitamins, and if vitamins stimulate the growth of Rhizobium, then it is possible that the stimulation may have been due to the vitamin content of the yeast extract. Werkman (18), from a study of the effects of vitamins on the growth of Rhizobium and Azotobacter, came to the conclusion that the stimulation was due to the additional amount of energy material added to the cultures and not to the vitamin content of the material added. His work would indicate that the stimulation of Rhizobium by the yeast extract was due to some factor other than auximones or vitamins.

Furthermore, it is entirely possible that some mineral constituent of the yeast extract was the stimulative agent. The effects on the growth of Rhizobium of small amounts of such elements as manganese, boron, and others that in the past have not been considered essential for plant growth, have not been investigated.

It is clearly evident that something other than the nitrogen content of the yeast extract may have stimulated the growth of Rhizobium in these experiments. Further work has been planned and is now under way to study the factors controlling this stimulation, with special emphasis upon the effects of the nitrogen factor alone. The results of this work will be presented in later publications.

SUMMARY AND CONCLUSIONS

Experiments have been conducted to study the effects of nitrogen of different sources and in various concentrations upon the growth of *Rhizobium leguminosarum*. The effects were interpreted from changes in the rate of oxygen consumption as measured in respiration chambers connected with micromanometers of the type used by Warburg.

In the media without nitrogen the organisms continued to respire at a low uniform rate throughout the various tests. Presumably they were in the resting state, and no growth occurred in the cultures.

In a similar medium except where nitrogen was added in the form of bacto-yeast-extract, the growth of Rhizobium was stimulated almost in direct proportion to the amount of the extract added. The rate of oxygen consumption increased during the first 12 hours, after which it remained constant or decreased slightly. Presumably after the organisms had utilized all of the nitrogen in the culture, or after they had passed the logarithmic phase of growth they were in the resting state, and no growth occurred.

In media containing nitrogen added in the form of ammonium chloride, sodium nitrate, urea, or alanine, there was no marked stimulation of growth irrespective of the concentration of nitrogen in the medium, and presumably the cells remained in the resting state throughout the experiment. There is some evidence that oxygen consumption by the organisms was depressed by ammonium chloride and also by the urea in the higher concentrations employed.

Whether the stimulation of growth induced by the yeast extract was due to its nitrogen content or to some other constituent of the extract is not known. It is entirely possible that the particular form or combination of nitrogen in the yeast extract is especially suitable for use by Rhizobium. It is also possible, however, that the stimulation of growth was induced by energy furnishing material, accessory food constituents, or certain elements necessary for the growth of the organisms which were not supplied in the basic medium.

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PLATE 1

Apparatus for Measuring the Effects of Nitrogen on Rhizobium

Fig. 1. Respiration vessel. The culture of bacteria may be seen in the bottom of the vessel and the KOH in the inside tube.

Fig. 2. Simple manometer of the Warburg type with respiration vessel attached.

Fig. 3. Water bath in which the temperature of the water surrounding the respiration vessels was thermostatically controlled within 0.01°C.

FIG. 1

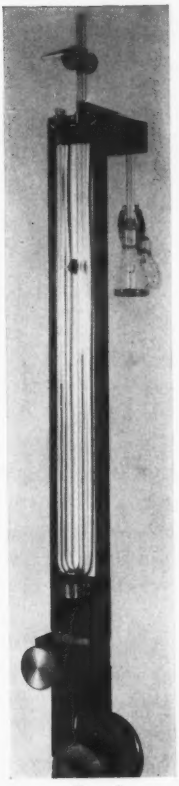

FIG. 2

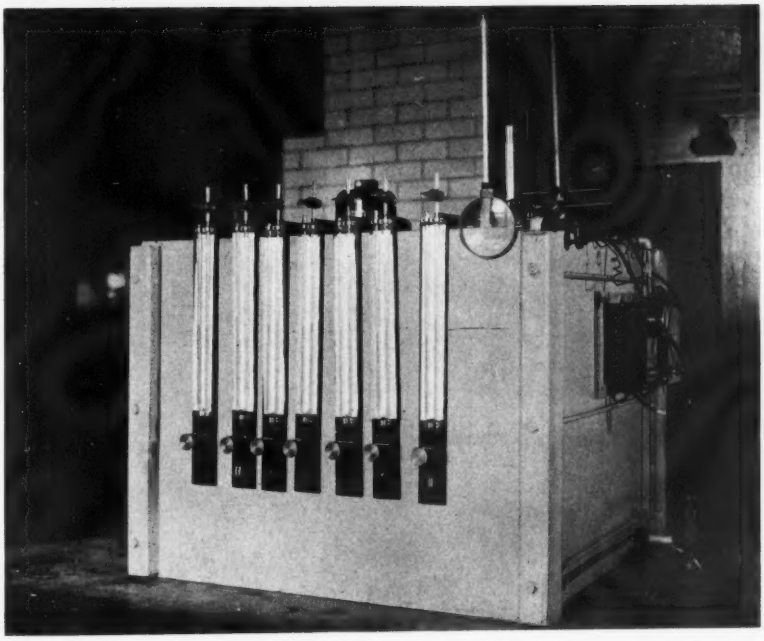

FIG. 3

LAWS OF SOIL COLLOIDAL BEHAVIOR: ~~XIV~~ I. AGING OF COLLOIDS AND BASE EXCHANGE[1]

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The reactions shown by many complicated materials such as the soil, silicates, and complex salts, are very difficult to represent by any simple dissociation formulas. Thomas and Whitehead (16) obtained results with aluminum oxychloride, which could be interpreted on the Werner theory, but for which the ordinary dissociation equations were inadequate to explain the observed facts. A similar explanation had previously been advanced by Bjerrum (1) to account for the increase in molecular weight of basic chromic sulfate on aging. Their explanation for both the chromic and the aluminum salt is identical, and the change that takes place is represented as follows:

$$\begin{bmatrix} H_2O & & OH \\ H_2O & Cr & H_2O \\ H_2O & & H_2O \end{bmatrix} \quad 2Cl + \quad \begin{bmatrix} OH & & H_2O \\ H_2O & Cr & H_2O \\ H_2O & & H_2O \end{bmatrix} \quad 2Cl$$

$$\rightarrow \quad \begin{bmatrix} & OH & \\ (H_2O)_4\,Cr & & Cr\,(H_2O)_4 \\ & OH & \end{bmatrix} \quad 4Cl + 2H_2O$$

the two hydroxyl groups being combined through residual valencies to give a larger molecule. This process, termed "olation," accounts for the increase in molecular weight and for the diminished activity of the hydroxyl groups.

Stiasny (15), in order to interpret the increased acidity and the decreased solubility of chromic salt on aging, made the second assumption that the hydrogen wandered from the oxygen, giving rise to "oxolation," with the secondary formation of a true valence bond between the oxygen atoms and the metallic element:

$$\left[(H_2O)_4\,Cr\begin{matrix}OH\\OH\end{matrix}Cr\,(H_2O)_4\right] 4Cl \rightarrow \left[(H_2O)_4\,Cr\begin{matrix}O\\O\end{matrix}Cr\,(H_2O)_4\right] 2Cl + 2HCl$$

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[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department soil chemistry and bacteriology.

The work reported in this paper was done in conjunction with the researches of Sante Mattson, to whom the author is indebted for guidance and encouragement. ~~It is for this reason that the paper appears in Mattson's series "Laws of Soil Colloidal Behavior."~~

[2] Formerly British Ministry of Agriculture Research Scholar, stationed at Rothamsted 1928–1931.

The object of this investigation was to extend this work and to apply the results, so obtained, to the investigation of base exchange. The effect of aging was studied with both aluminum hydroxide and with aluminum silicate, and a different theory based on atomic structure is advanced to account for the results, which substantiate the previous investigations of other workers.

PREPARATION OF ALUMINUM HYDROXIDE SOL

Ten grams of aluminum hexahydrate was dissolved in CO_2-free water, and aluminum hydroxide was precipitated with sodium hydroxide until flocculation commenced, the hydrogen-ion concentration of the supernatant liquid being adjusted to the isoelectric pH 8.2. The sol was centrifuged, the supernatant liquid decanted, CO_2-free water added, and the process repeated until a little peptization was observed. The floc was subsequently transferred with distilled water to a flask and protected from CO_2. Twenty cubic centimeters of the suspension was placed in boiling tubes, and the pH determined in each one at intervals with the quinhydrone electrode, at a temperature of 25°C.

TABLE 1

Effect of aging on pH of aluminum hydroxide

	TIME IN DAYS													
	0	1	2	3	4	5	6	7	8	9	10	11	12	40
pH	7.31	7.11	6.35	6.15	5.90	5.90	5.67	....	5.65	5.60	5.61	5.33	5.33	4.67

Equilibrium was usually reached in 30 minutes. The results are given in table 1.

The increase in hydrogen-ion activity with time is surprising, and at the end of 40 days the pH was only 4.67. Although the interpretation on Werner's theory is sufficient to explain the results, the original idea of Werner has been superseded by the development of theories of valency based on atomic structure, and for the interpretation of results obtained with silicates, the theory is much more advantageous. A brief review of the modern conception of atomic structure, all that is required to discuss the results obtained, is given below, but for greater detail the reader is referred to Sidgwick (4).

ELECTRONIC INTERPRETATION OF VALENCY

Prior to 1913, there existed no very definite relation between structure and valency, but at this time atomic numbers were discovered. This gave the number of electrons in each atom and indicated that the numbers in the inert gases were capable of a very stable arrangement. Kossel (6) pointed out that the element before an inert gas is a strongly electronegative halogen, and immediately following occurs a strongly electropositive alkali metal. These ele-

ments would then combine because of their tendency to reach the stable arrangement of an inert gas. Thus in the series

S	Cl	A	K	Ca
16	17	18	19	20

eighteen electrons must be capable of a very inert and stable arrangement since argon contains this number. If potassium would lose one electron to the chlorine, both would achieve this stable number, and calcium could do so by giving 2 electrons to 2 chlorine atoms. This would give, on dissociation of the compound, the stable ions Cl, K, Ca, all with 18 electrons, the chlorine ion with one more than necessary to balance the charge in its nucleus, the potassium with one less than necessary to balance the charge on its nucleus. On this theory the valency of an atom is the number of electrons it must gain or lose in order to reach the stable number of an inert gas, but the elements would have to be utterly dissimilar in character.

There are molecules in which it is impossible to distinguish between the positive and negative atom, as when two hydrogen atoms combine to form a molecule, and on Kossel's theory such a combination could not be accounted for. The solution to the difficulty was given by Lewis (8), who, like Kossel, assumes that the cause of chemical combination is the tendency of electrons to distribute themselves so as to reach the stable arrangement of the inert gases, but he made a further assumption of great importance, that is, that atoms can also share electrons so that the shared electrons contribute in some way to the stability of both atoms. Chlorine (17)[3] has one electron less than the stable number of argon (18). Hence, it readily takes up one from an atom like potassium (19) to form potassium chloride, a compound which readily ionizes in solution and is also ionized even in the solid state. It can also complete its number of electrons by combining with another chlorine atom, if it shares one electron belonging to another chlorine atom; and if at the same time the second chlorine atom shares an electron belonging originally to the first, then each is satisfied. The Lewis method permits a smaller number of electrons to do the work of what on the Kossel model would require more. To distinguish these two forms of linkage Langmuir suggested the terms "electro-valency" and "co-valency," the former giving the linkage in potassium chloride, the latter in molecules such as molecular chlorine or hydrogen.

The necessity of two shared electrons to every co-valent link forms the basis of Lewis's theory, the process being the same as the linking of hooks, so that the molecule is, as it must be, electrically neutral. The single link between potassium and chlorine, or between two chlorine atoms, both satisfies the single valency of potassium and of chlorine, in both cases, and gives the stable member of an inert gas. Thus the theory of Lewis provides a mechanism for two kinds of linkage, "polar" and "non-polar," which the chemical evidence requires.

[3] The number in parentheses immediately following the name of the element refers to its atomic number on electronic arrangement.

No atomic model is involved in the theories, but subsequently Bohr advanced a theory that electrons are distributed in shells around the proton nucleus, each shell corresponding to a definite energy level. In hydrogen the kernel of the atom is the nucleus itself, and it has one valence electron. The two electrons of helium are not regarded as valence electrons, and it is considered that the whole of the atom represents the kernel. The pair of electrons in helium constitute the shell, and a similar pair will appear as the first shell of all atoms of higher atomic numbers. The atomic number and kernel of the atoms reqiured for this discussion are given in table 2.

The electrons in the outer shell are the valence electrons. Thus hydrogen can share its electron to reach the stable number of helium by combining with

TABLE 2

Atomic number and kernel of various atoms

ELEMENT	ATOMIC NUMBER	KERNEL OF ATOM GROUPS 1 2 3 4
H	1	1
He	2	2
Li	3	2 + 1
Be	4	2 + 2
B	5	2 + 3
C	6	2 + 4
N	7	2 + 5
O	8	2 + 6
F	9	2 + 7
Ne	10	2 + 8
Na	11	2 + 8 + 1
Mg	12	2 + 8 + 2
Al	13	2 + 8 + 3
Si	14	2 + 8 + 4
A	18	2 + 8 + 8
Fe	26	2 + 8 + 13 + 3

chlorine (2) (8) (7), whereas lithium combines with chlorine if it loses one electron to reach the stable number of helium. Sodium can lose its valence electron if it combines with chlorine, the sodium achieving the number in the next gas neon (2) (8), the chlorine by gaining one reaching the number in argon (2) (8) (8). Helium with only a pair of electrons has the same stability as the remaining rare gases with an outer shell of eight, the octet. It was therefore originally supposed that the redistribution of electrons on chemical combination led to the production of an outer group of eight, with all atoms except the immediate neighbors of helium, where the number is two. This was the foundation of the octet theory of Lewis, on a cubic model of the atom. Although the "static atom" has been abandoned, it is still true that the stability of a large number of compounds is determined by their having in combination a group of eight shared electrons, and since a pair of electrons constitutes a single bond, a co-valency of four.

On this basis a mechanism is given for two kinds of linkage:

(*a*) Polar or ionized linkage, as in potassium chloride, due to the transference of an electron from one atom to the other.

(*b*) Non-polar, non-ionized linkage (co-valency), due to the sharing of electrons two to each link as in the combination of atoms to form molecules.

Both are represented by two dots, to represent a single bond:

(*a*) K—Cl K:$\dot{\underset{\cdot\cdot}{\text{Cl}}}$:

(2) (8) (1) (2) (8) (7) (2) (8) (2) (2) (8) (8)

(*b*) H—H H:H

(1) (1) (2) (2)

And in combination the shared electrons contribute to the stability of both atoms, reaching the inert gas number.

It should be noted that hydrogen achieves stability with only two shared electrons. The chlorine in potassium chloride achieves stability by completing the octet or its outer shell; the outer shell of the potassium however consists of only two shared electrons. In order to maintain the octet theory, it has been suggested that the inner shell of the potassium be regarded as the octet. It is unnecessary however as such compounds are never really combined, the group (2) (8) (2) being almost always a highly ionized compound, such as the strong electrolytes.

There is another mode of combination of very great importance and first suggested by Lewis. A divalent electronegative atom like oxygen (2) (6) achieves stability on combination by gaining electrons to reach the stable number of neon (2) (8). It can gain these by forming two links, that is, by sharing two electrons belonging to another atom or atoms while the other atom shares two belonging to the oxygen:

$$R\ :\dot{\underset{\cdot\cdot}{\text{O}}}:\ R \quad \text{or} \quad R—O—R$$

This is the usual divalent oxygen of structural chemistry. The new mode of combination suggested by Lewis is that oxygen forms only a single co-valency, shares only two electrons with the other atoms, but that both of these come from the other atoms:

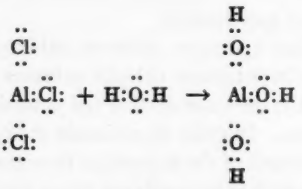
$$R: + \dot{\underset{\cdot\cdot}{\text{O}}}: \rightarrow R\ :\dot{\underset{\cdot\cdot}{\text{O}}}:$$

This constitutes only a single link, but it increases the valency electrons of the oxygen by two, and it becomes saturated, achieving the number of the inert gas neon. Lewis' suggestion is that two shared electrons which constitute a co-valency can come in two ways: (*a*) as they usually do, by each of the atoms contributing one of them; or (*b*) by one of the atoms contributing both. The

latter is termed a "co-ordinate link" or "semi-polar bond," and gives an explanation of the co-ordination compounds of Werner. It gives peculiar properties to a molecule, and in particular a very strong external electrostatic field, but it is much more easily broken than an ordinary co-valency, and the compound can again be broken up into simple molecules. This is the type of linkage that apparently occurs in aluminum hydroxide, and probably in the clay fraction of the soil, both aluminum hydroxide and clay being susceptible to hydrolysis.

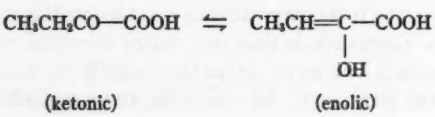
AGING OF ALUMINUM HYDROXIDE SOL

The formation of a hydroxide from a chloride such as aluminum chloride is a hydrolysis, the preliminary reaction being the disssociation of chlorine, which is combined with aluminum by electro-valencies, and the compound being highly ionized:



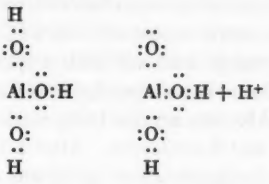
The Cl^- on dissociating takes away the original electrons of the aluminum (2) (8) (3), giving the Cl^- ions. The aluminum subsequently combines with the OH^- ion, but both the electrons come from the negative oxygen, whether from water or alkali. This is a co-ordinate co-valency, or a semi-polar link, on the theory as previously outlined, as both electrons necessary for the formation of a single link are obtained from one atom, the oxygen, of the hydroxyl ion. The aluminum is unsaturated, however, as it has not during the formation of the hydroxide achieved the stable number of the inert gas, that is, its outer group has only six shared electrons and not the eight of the inert gases. This phenomenon of atomic unsaturation is the electronic interpretation of Werner's residual valency.

Suspending the aluminum hydroxide in water leads to a rearrangement which can be easily interpreted on this theory. The hydrogen ion is the smallest ion known, and because of this it is particularly free from the "steric" effects to which other larger ions are susceptible, as in the phenomenon of tautomerism that occurs in aceto-acetic ester, where the hydrogen wanders between the carbon and the oxygen, giving rise to two compounds of the same molecular weight:



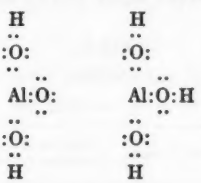
(ketonic) (enolic)

The first step that occurs is the wandering of hydrogen from a molecule (*A*) of the aluminum hydroxide to give a molecule (*B*):



(*A*) (*B*)

The oxygen will retain the pair of electrons, but a rearrangement will take place, by a molecule such as (*B*) combining with another molecule such as (*A*), the aluminum of molecule (*A*) achieving stability by combining with the lone pair of electrons of the oxygen of molecule (*B*), the aluminum of (*A*) thus becoming saturated, by a co-ordinate co-valency giving molecule (*C*).



(*C*)

The molecular weight thus consistently increases and the process is capable of an indefinite formation, the molecule always remaining unsaturated, or it may possibly go through a rearrangement to give a complicated ring structure after some time. Since the pH of the suspension apparently diminishes consistently with time, the latter is improbable. The formations of the bonds always arise through a single linked oxygen —O— and not O=, and would be difficult to represent on any theory other than on that based on atomic structure, which gives a reasonable explanation of both the increase in molecular weight and the increased acidity of the suspension, the hydrion combining with the remaining salt, sodium chloride, giving hydrochloric acid, and, because of the internal rearrangement of the molecules of hydroxide itself, there is no place for it again to re-enter the molecule, the electrons of the oxygen attaching themselves to the unsaturated aluminum.

AGING OF ALUMINUM SILICATE AND ADSORPTION OF BARIUM BY THE COLLOID ON AGING

Although the interpretation of the data for aluminum hydroxide is comparatively easy, the chemistry of silicates, which is of the utmost importance for soil workers, is much more complicated. In order to study the phenomena of aging with aluminum silicate, the compound was prepared from the chloride

by mixing aluminum chloride and sodium silicate and adding sodium hydroxide until flocculation commenced. The floc was centrifuged and washed with CO_2-free water until a slight peptization was observed, as with the hydroxide, and kept in distilled water in a bottle protected from CO_2. The pH of the suspension was determined at weekly intervals with a quinhydrone electrode, and simultaneously 50 cc. of the sol was suspended in 100 cc. of barium acetate, the total volume of the sol and barium acetate being made up to 200 cc. for a study of the barium adsorption and flocculation. After a time the sols were centrifuged, and the barium in the supernatant liquid was determined. The difference between the original barium content of the barium acetate and that in the supernatant liquid was taken as being the quantity of adsorbed barium. The results are given in table 3.

The sol thus shows the same effect of aging that was evident with aluminum hydroxide, the pH has a tendency to decrease and shows a considerable diminution the first week. This phenomenon will be discussed first, and the second effect of diminished adsorption on aging will be discussed later.

TABLE 3

Effect of aging on the pH, and adsorption capacity of aluminum silicate

50 cc. sol = 3.02 gm.

AGE	pH OF SOL	Ba^{++} ADDED	Ba^{++} IN SUPERNATANT LIQUID	Ba^{++} ADSORBED	Ba^{++} ADSORBED BY FRESH SOL
days		*m.e.*	*m.e.*	*m.e.*	*m.e.*
0	4.61	11.055	9.408	1.647	100
7	4.52	11.055	9.824	1.231	74.7
14	4.52	11.055	9.976	1.079	65.5
21	4.52	11.055	10.088	0.967	58.7

The normal state of combination in a molecule is reached when each atom of hydrogen has its stable pair of electrons, as in helium, and each other atom the stable group of eight, with the exception of the highly dissociated compounds such as potassium chloride, where even then the inner group can be taken as the octet. A combination of (2) (8) (2) is, however, always highly ionized. Molecules are achieved by atoms sharing between them a pair of electrons that constitute a single bond. In a double bond formation this, however, cannot occur, as in the combination of atomic oxygen to give the molecule. Oxygen (2) (6) has six valence electrons and requires a share in two more in order to complete its octet. Even if two atoms share an electron pair, this does not complete the octet of the two atoms. If, however, two electron pairs are shared, then each atom can be said to have its outer group of eight $:\ddot{O}: :\ddot{O}:$, the four shared electrons now counting independently toward the stability of both atoms and giving the double bond O = O.

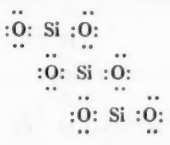
Double bonds, such as those for oxygen, occur often with carbon compounds.

A consideration of the great difference in physical properties between the compounds of carbon and of silicon, which have the same valency [C (2) (4), and Si (2) (8) (4)], led Lewis (8) to suggest that multiple bonds were confined to the elements of the first period of eight, and especially to carbon, nitrogen, and oxygen. Carbon dioxide and silicon dioxide are totally unlike in physical properties, and the two formulas, O = C = O and O = Si = O, in no way account for the great dissimilarity in the physical properties of the two substances.

If silicon is incapable of forming double bonds, then in terms of the older valence theory, silicon dioxide would have to be represented as

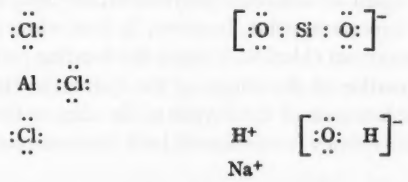
—O—Si—O—

and the uncompleted bonds could be completed only by union with other molecules. In terms of the newer theory, it would be represented as follows, a pair of electrons forming a single link :Ö: Si :Ö: With such a structure the oxygen has a lone pair, and the silicon only four, instead of the eight required for stability. The molecules would combine to form larger molecules as of the following type:



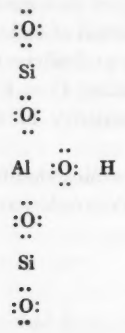
In such a formula the existence of unsatisfied valences is indicated, and the process of polymerization would go on indefinitely until all the material was exhausted, as with the aging of aluminum hydroxide sol.

When sodium silicate is added to aluminum chloride, the following ions are in the solution:

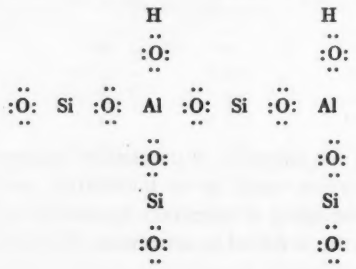


and as the electrovalent chlorine dissociates, the silica or the hydroxyl ion will become attached to the aluminum through the lone pair of the oxygen, the two electrons necessary for the formation of a bond being given by one and the same atom, thus constituting a co-valency. The following formula is given as

an illustration of the type of bond, but the two oxygen atoms of the silica may be giving their lone pairs of electrons to the aluminum:



A compound of this structure is highly unsaturated, the aluminum having only six shared electrons and the silicon only four. In order to reach the stable group of eight, the molecules will polymerize, union taking place between the molecules either at the silicon or aluminum atoms, through the oxygen of the silicon fraction or through the oxygen of the hydroxide group, after the H ion dissociates, giving a compound of the following type:



which is capable again of indefinite polymerization until all the material is exhausted. The important point, however, is that when the chlorine dissociates from the aluminum chloride, it takes the bonding pair of electrons with it, and for the formation of the silicate or the hydroxide, the bonding pair are contributed by the lone pair of the oxygen of the silica or hydroxyl ions. This is by definition a co-valency (co-ordinate), both electrons coming from one and the same atom.

A clear distinction must be drawn between a co-valency and an electro-valency, the normal co-valence that exists between like atoms to form molecules being neglected. Why there should be so great differences in properties of compounds, one with an electro-valence and the other a co-valence, cannot adequately be answered. That they are two different forms of linkage is, however, upheld by Sidgwick (14). In an ionized compound, such as potassium

chloride, which results from the transference of an electron from the potassium to the chlorine, thus,

K	Cl	K	Cl	K⁻	Cl⁻
(2) (8) (8) (1)	(2) (8) (7)	(2) (8) (8) (2)	(2) (8) (8) (2)	(2) (8) (8)	(2) (8) (8)

with subsequent dissociation, a true bond, it is believed is never formed, the ions being held together purely by electrostatic attraction, and even in the solid state the potassium chloride is ionized. In co-valent compounds, however, the links form part of the constitution of each atom in the molecule, and co-valencies, unlike electro-valencies, are directed forces, and beyond limits of slight distortion their position in space, like those of carbon compounds, is fixed, and it is quite possible to have the same groups arranged around the same atom in more ways than one, giving rise to structural isomerism. The conditions of when a co-valency changes into an electro-valency will be discussed when we come to base exchange.

The distinction was first drawn by Werner, with compounds formed by metallic salts. He showed that in the platinum amines, the platinum is at the center of a co-ordination zone, in which a number of atoms are attached to the central atom, and this number he called the co-ordination number. Those atoms within the co-ordination shell, represented by brackets, were supposed to be bound by true valences and were ionized linkages. Thus in the following series of compounds with platinum:

$$\left[\begin{matrix}\text{Pt} & (\text{NH}_3)_4\end{matrix}\right]\text{Cl}_2 \quad \left[\begin{matrix}\text{Pt} & (\text{NH}_3)_3 \\ & \text{Cl}\end{matrix}\right]\text{Cl} \quad \left[\begin{matrix}\text{Pt} & (\text{NH}_3)_2 \\ & \text{Cl}_2\end{matrix}\right] \quad \left[\begin{matrix}\text{Pt} & \text{Cl}_4\end{matrix}\right]\text{K}_2$$

as the NH_3 is replaced by Cl, the conductivity of the compounds diminishes. And the third compound was found by Werner to be a non-electrolyte. On further substitution of the NH_3 by Cl, the charge of the compound changes from the original of a double positively charged particle to a double negatively charged particle. All the ions or radicals that can enter a co-ordination group have a lone pair of electrons as the chlor ion $:\ddot{\text{Cl}}:$, or ammonia, the nitrogen of which has a lone pair

$$\begin{matrix} & \text{H} & \\ & \ddot{\ } & \\ \text{N}: & & \text{H} \\ & \ddot{\ } & \\ & \text{H} & \end{matrix}$$

The original distinction between valences as drawn by Werner, indicated by the terms "true" and "residual" valences, vanishes. Those within the first shell are, as a matter of fact, co-ordinate links, the outside atoms being held purely by electrostatic attraction. The distinction thus vanishes, and the valences that unite the groups to the central atom in Werner's co-ordination zone are identical co-valences, whether with an atom like chlorine or a radical like ammonia, the bond being formed by a lone pair of electrons.

A co-valency, or the number of co-valencies that an atom can form, is not conditioned by its position in the periodic table. In the aluminum hydroxide the aluminum is originally tri-valent, has six electrons obtained from the hydroxyl groups. It is, however, still unsaturated and requires eight for stability, and on aging it obtains two more as the hydrion dissociates from the hydroxyl group, and the oxygen attaches to the aluminum its lone pair. The aluminum inside the compound has thus increased its valency from three to four.

AGING AND STABILITY OF ALUMINUM SILICATE SOL

After the work of Hardy (3), it was believed and has since been frequently stated, that flocculation of colloid particles is due to the neutralization of the charge on a colloid by an oppositely charged ion. If it be assumed that the stability of a sol is due to the potential difference between two concentric layers, the sol will be more stable the higher the potential difference, and if the stability depends solely on the neutralization of the charge, the greater the adsorption of an oppositely charged ion, the greater the flocculation of the colloid. This view that the stability of the colloid is in some way connected with the cataphoretic potential is widely accepted, and the colloid on this viewpoint is least stable at its isoelectric point. Mattson (9), however, found that the flocculated colloid had in some cases a higher cataphoretic mobility than the sol from which it had been formed by coagulation with electrolytes.

The aluminum silicate synthesized as described was strongly positively charged, that is, the pH 4.5 was below the isoelectric pH of the compound, and it dissociated Cl ions. On suspension in barium acetate the pH came to pH 7.0, and excess of Ba^{++} was in the solution, so that actually, in studying the flocculation in barium acetate solution, we are dealing with the neutralization of a colloid that is negatively charged. The cataphoretic mobility in barium acetate could not, however, be determined, as the polarization at the poles of the cell makes such a determination impossible, but a pH 7.0 is considerably higher than the isoelectric pH of any aluminum silicate studied in this laboratory. It is therefore reasonable to suppose that the colloid was dissociating cations and would thus be negatively charged at such a high pH.

The difference in flocculation with the age of the colloid in a solution of barium acetate is very striking. The flasks were photographed by transmitted light and show clearly the difference in the stability with the age of the colloid, the oldest being the least stable, and a clear field is obtained with transmitted light, although it adsorbed the least amount of ions of opposite sign of charge. The increased flocculation could hardly thus be in any way due to neutralization of the charge by an ion of opposite charge (plate 1).

The increased coagulation is, however, due to the increased size of the particles, falling in the barium acetate under the action of gravity, for all the particles contain adsorbed barium in the external layer, and the particles themselves, once the barium has combined, have the same sign of charge. If the theory for aging is correct, and polymerization due to unsaturation results,

then this increased coagulation due to increased size of particles ought to be expected on Stokes' law, which states that if the force is gravity then $V = Cr^2$, the limiting velocity with which particles fall in a liquid of the same viscosity is proportional to the square of the effective radii. It should be noted that the Ba^{++} prevents the aging as judged from flocculation, so that a particle of Ba-colloid is actually smaller than that of a H-colloid.

BASE EXCHANGE

The phenomenon of base exchange was first discovered by Way (17), who percolated a solution of KCl through a soil and found that a differential adsorption takes place, the potassium being retained and another element, mostly calcium, coming into solution. The reaction may be expressed as:

$$\text{Soil} - \text{Ca} + 2\text{KCl} \rightarrow \text{K}_2 - \text{Soil} + \text{CaCl}_2$$

Acid soils under similar circumstances exchange H ion for the K^+ ion, and the reaction may be expressed as:

$$\text{H} - \text{Soil} + \text{KCl} \rightarrow \text{K} - \text{Soil} + \text{HCl}$$

These reactions are termed base exchange reactions and have the advantage of simplicity of representation. The exact nature of the process, however, requires a great deal of elucidation. It has been supposed that the cations are held at the surface of a colloidal anion as in the Helmholtz double layer and can then be replaced by the cations of a salt without altering the stability of the particle.

Mattson (9) proceeded to elucidate base exchange reactions for materials other than soil, such as hydroxides of iron and aluminum, phosphates, and silicates. Basing the theory on the conception of amphoteric electrolytes, he determined in each case the pH at which the various compounds are isoelectric. Ferric hydroxide prepared from the chloride is isoelectric at pH 7.1 and the corresponding aluminum compound at pH 8.2. If these compounds are leached with an electrolyte, the pH of which is equal to their isoelectric pH, such as a solution of normal barium acetate, no base exchange occurs, or an extremely small amount. If they are treated with a salt solution the pH of which is higher than the isoelectric pH, base exchange occurs, but if treated with a solution the pH of which is lower than the isoelectric pH, anion exchange occurs, that is, the hydroxyl ion is replaced by the anion of the salt, and not the hydrion by the cation of the salt. The isoelectric point thus assumes a new significance. It is not only the point of least stability of a sol, but it is a very significant point from the viewpoint of ion exchange. It is defined as the point at which the colloid is discharged, but characterized as well by the determination of pH at which the discharge occurs.

On the theory of amphoteric electrolytes, the isoelectric pH is given by

$$H = \sqrt{\frac{Ka}{Kb}\ Kw}$$

where Ka = dissociation constant of acidoid component;
Kb = dissociation constant of basoid component;
Kw = dissociation constant of water

and the isoelectric pH will depend on whether the acid or basoid component is strongest, that is, has the strongest dissociation.

This theory of dissociation tendency, with suitable modifications, has a great deal in common with the usual theory of dissociation for the soluble electrolytes. The following exposition can be given, silicates being taken as an example. The anion of silicic acid is known as a sluggish ion with small mechanical mobility, although it may have a high electrical mobility. Its dissociation tendency is the same as that of any other acid, but the resulting anion does not become molecularly dispersed but remains bound in colloidal aggregates. If a particle of soil is placed in water, its dissociation tendency is made evident by the lowering of the pH, that is, it splits off H ions at its surface, which remain as the surface layer surrounding the anion, being held by electrostatic attraction. The charge of the colloid particle thus arises from dissociation, and an analogy can be made with any other acid thus:

$$[\text{Soil}] - H \rightarrow [\text{Soil}] + H$$
$$CH_3COOH \rightarrow CH_3COO + H$$

If the colloid is amphoteric, that is, if it acts both as a base and as an acid, it must dissociate in two separate ways:

$$XOH \rightarrow [XO]^- + H^+$$
$$\rightarrow [X]^+ + \overline{OH}$$

At the isoelectric point, if the charge arises out of dissociation of ions, the colloid is isoelectric because both dissociations are equal. It is important in this theory to realize the obvious fact that both dissociations are equal when there is no dissociation at all.

Chemical reactions take place between ions partially bound or free. By partially bound ions we mean those held at the surface of another ion by electrostatic forces, such as occur in a soil. These also occur in the solution of an electrolyte, where in the neighborhood of any ion there are more ions of an unlike charge than of the same charge. In the Werner salts, such as $\left[\begin{matrix}Pt(NH_3)_2\\Cl_2\end{matrix}\right]$ where the chlorine is bound by co-valencies, it gives no test with silver nitrate but does if the compound is $\left[\begin{matrix}Pt(NH_3)_3\\Cl\end{matrix}\right]Cl$. If base exchange is an ionic reac-

tion, as of course it is, and if a compound shows no base exchange capacity when leached with a solution the pH of which is the same as its isoelectric pH, then we are left with no alternative but to state that at its isoelectric point it is not ionized—it is neither cationic nor anionic. From the qualitative point of view, the test is identically the same as testing for Cl^- in Werner salts with silver nitrate. Since at this point there cannot be any ions dissociated from the colloid, it must, on the basis of the atomic theory, be a co-ordinate compound held by co-ordinate valencies, but we have to define the conditions under which a co-valency changes into an electro-valency.

In determining base exchange, such as with any colloid, by leaching with a normal solution of barium acetate, e.g.,

$$\begin{bmatrix} X \end{bmatrix}^{H}_{H} + Ba(OOCCH_3)_2 \rightarrow [X]\,Ba + 2CH_3COOH$$

the reaction is thrown absolutely in one direction, by removal of the product of the reaction by leaching, so that the test for dissociation is extremely rigid, and yet neither ferric nor aluminum hydroxide, isoelectric at pH 7.1 and 8.2 respectively when prepared from the chlorides, shows any trace of base exchange when leached with a normal solution of barium acetate pH 7.0. There is, therefore, at this pH no cationic dissociation of these two colloids.

Co-valencies can, however, change into electro-valencies, that is, ions can dissociate from the compound under certain conditions, and for the systems studied the pH appears to be the determining factor. If an amphoteric colloid be treated with a salt solution at various pH values, it has been found by Mattson and Hester (10) that it dissociates H ions and lowers the pH of a salt solution which was originally above the isoelectric pH, that is, it dissociates in two ways:

$$X{:}\ddot{O}{:}H \rightarrow \left[X{:}\ddot{O}{:}\right]^- + \overset{+}{H} \text{ (pH above isoelectric pH)}$$

$$\rightarrow [X]^+ + \overline{OH} \quad \text{(pH below isoelectric pH)}$$

but in the sense that not all of the surface layer splits off H or OH ions. One may speak, therefore, of partial, or degree of, dissociation, one of the ions resulting from the dissociation, the anion, being incapable in the case of soil of achieving the molecularly dispersed state. Depending on the character of the dissociation, it should be observed that the sign of the charge of the colloid particle changes. In a salt solution the dissociated ions H^+ or OH^- are replaced by the ions of the salt, either partially or completely depending on the concentration of the salt.

Since the nature of the dissociation varies with the pH of the external medium, it would be expected that not only the nature but the amount or degree of dissociation should also vary. That is, if an amphoteric colloid is isoelectric at pH 6.0, by virtue of mass action, the dissociation of hydrion from

the complex would increase the higher the pH or the lower the hydrogen-ion concentration of the liquid phase, and the greater would therefore be the base exchange, the higher the pH of the salt above the isoelectric pH. The reverse would be true of anion adsorption, that is, the lower the pH of the electrolyte, the greater would be the dissociation of OH ions and the greater the replacement by other anions. This has been proved for a number of hydroxides, and the results will be published later. In order to illustrate the point, however, a sample of electrodialyzed bentonite (1 gm.) was treated with 4.0 millimols of $FeCl_3$, and the pH was brought by the addition of alkali to the isoelectric pH 6.5. The sample was freed from the mother liquor and the base exchange of the ferric bentonite was determined on 0.5 gm. of the dried sample by leaching with solutions of barium acetate at pH 6.0 and pH 7.0, the barium being subsequently replaced by NH_4 by leaching with normal ammonium chloride. At pH 6.0 the exchangeable barium was 0.192 m.e. per 0.5 gm., but at pH 7.0 it was 0.253. Since the isoelectric pH was at 6.5, if base exchange is regarded as a test for hydrogen ions there appears to be a marked contrast in the degree of dissociation at the isoelectric point for different amphoteric electrolytes. In the case of the hydroxides the minimum dissociation at the isoelectric point has a negligible value; with the silicated compounds it appears to reach a considerably higher value.

The position of the hydrogen ion in base exchange

Hydrogen occupies a unique position, as its kernel is composed merely of the nucleus with one planetary electron. Its stable shell in combination is not a group of eight, but a group of two, characteristic of the inert gas helium.

It has been found by Jenny (5) that with soil colloids the hydrogen ion is the most strongly adsorbed and the most difficult to replace. The order of adsorption of the various cations of the same valency at equivalent concentrations is as follows:

$$Li < Na < K < Rb < Cs < H$$

and for their release: $Li = Na > K > H$.

The strong adsorption of the H ion, which is attributed to its small volume, is surprising, for in all other branches of chemistry the high mobility of the H ion is attributed to its small volume, and in colloids, on aging, the whole phenomenon is interpreted on the basis of its mobility. Its strong adsorption and its mobility cannot therefore be attributed to one and the same cause, namely, its small atomic volume. It must be united by forces of a totally different nature from that which unites other cations, and although on the older theory of valency this cannot be explained, on the new it can, only one assumption being made, that is, that a chemical bond is a much stronger force than an electrostatic attraction between oppositely charged ions.

It has been shown that the surface, the seat of base exchange, in both hydroxides and silicates, consists of hydroxyl groups, or oxygen atoms with a lone

pair of electrons. The H ion dissociates little at the isoelectric point but the colloid cast as an acid if treated with a salt solution the pH of which is above its isoelectric pH, as a base if the pH is below its isoelectric pH, the dissociated ions forming a diffuse layer surrounding the colloid ion, and held there by electrostatic attraction. That is, the co-valent bonds change into electro-valent bonds at a pH removed from the isoelectric pH, but at two different points in the compound.

The strong adsorption of the H ion has been proved by treating a soil colloid with the hydroxides of lithium, etc., and subsequently releasing the adsorbed cation with an equivalent amount of acid. All soil colloids studied thus far have their isoelectric pH below pH 7.0, so that if placed in an alkaline medium, with a low concentration of H ions, the dissociation of the hydrion will be nearly complete, but the subsequent replacement of the H ion in the diffuse layer will depend on the concentration of the salt under equilibrium conditions. If this were not so, when a colloid is leached with a salt solution at any pH, the base exchange ought to go to completion whatever the pH, and since it does not, then replacement of the H ion of the diffuse layer is the secondary reaction, the primary reaction being the degree of dissociation of the H^+ ions which is a function of the pH and of the pH alone.

If the colloid is represented by $X :\ddot{O}: H$, it will dissociate in an alkaline medium into $\left[X :\ddot{O}:\right] + H^+$, the hydrion being replaced by a cation, but the oxygen retains the bonding pair of electrons. On the addition of acid the hydrion with no attendant electrons has in the lone pair of the oxygen the exact number that it requires to form a true chemical bond and reach the stable number of helium (2). No other cation can form a true chemical bond other than the hydrion. Its small volume has nothing to do with its strong adsorption, for it forms a true co-valent bond.

The weakly adsorbed bases Na, K, Mg, Li, Ca

Soil colloids in their natural state are always electronegative, that is, dissociating cations from the surface, and to determine their isoelectric point they must first be electrodialyzed, which can be looked upon as a hydrolysis with the addition of external energy:

$$\text{Soil} - \text{Na} + \text{H} - \text{OH} \rightarrow \text{Soil} - \text{H} + \text{NaOH}$$

Since they are electronegative, the pH of the soil solution must be actually higher than the isoelectric pH of the soil colloid free from exchangeable bases. Under such conditions the soil colloid gives an acid dissociation, that is, the H ion dissociates, the co-valent bond between the oxygen and the hydrogen being broken, the oxygen retaining the bonding pair of electrons. The ions of the various cations that occur in the soil solution have the following system of planetary electrons: Na (2) (8); K (2) (8) (8); Li (2); Ca (2) (8) (8). On

the basis of the newer conception of valency, they cannot form a true bond, that is, a co-valent bond. Thus if sodium ion combines with the oxygen we have the structure (2) (8) (2), characteristic of highly dissociated salts like sodium chloride but not a true bond. Cations other than hydrion require eight electrons for stability, and with a single bond oxygen, they cannot obtain them. Because of the charge on the oxygen atoms, the cations are held, therefore, purely by electrostatic attraction of the negative colloidal ion. The force of attraction will vary with Coulomb's law, being inversely proportional to the square of the distance between charged spheres of opposite sign of charge. The distance between them, however, will depend not on the natural atomic volume of the ions, but on the hydrated ions, and since it has been shown by Wiegner (18) that the smaller the ion the greater the sheath of water molecules, the greater will be the distance separating the smaller ion from the colloid anion, and the smaller will be the force of attraction and the greater the dissociation. It may be remarked that an ion that is strongly adsorbed must be weakly dissociated, for the force of attraction is identically the same for both adsorption and dissociation from the colloid. At different concentrations, however, we are dealing with a force of repulsion between the cations themselves as well as a force of attraction with the colloidal anion.

Influence of aging on the adsorption of barium by aluminum silicate

As the aluminum silicate ages, its capacity for adsorption of barium diminishes, and table 1 shows that after the silicate has aged for 3 weeks, the amount of barium adsorbed by the oldest colloid was only 58 per cent of the adsorption capacity of the young freshly prepared colloid. Whatever the ion at the surface, the force of attraction at the surface is due to the lone pair of electrons shared between the ions and the surface oxygen. A molecular rearrangement with the transference of these electrons to saturate the aluminum diminishes the surface forces and hence the degree of adsorption.

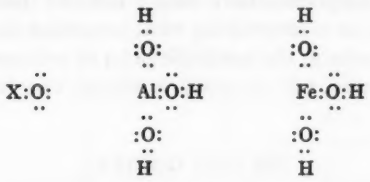
Iron and aluminum in base exchange

One gram of electrodialyzed bentonite in 1 liter of water was mixed with 4.0 millimols of $AlCl_3$ and $FeCl_3$ in a liter, and the pH of the supernatant liquid was brought to the isoelectric point pH 6.75 and pH 6.5 respectively. Complete adsorption of the iron and aluminum took place. The flocs were freed from the mother liquor, dried at 65°C., and leached with normal ammonium chloride, but no trace of iron and aluminum could be found in the leachate. Although base exchange was not done on these precipitates, the cation exchange capacity of similar compounds was determined by Mattson (9) and had the following values in milligram equivalents per gram at pH 7.0: original bentonite, 1.002; bentonite Al_2O_3 complex, 0.429; bentonite Fe_2O_3 complex, 0.970.

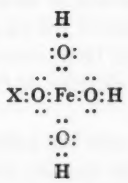
If these two ions do not take part in base exchange reactions, they cannot be at the surface of the colloidal anion but are blocked by some other ions. The atomic theory permits of an easy interpretation. On the assumption that

the bentonite has the same structure as that given the aluminum silicate, it can, as far as its surface is concerned, be represented by *X*OH, and since it is largely composed of aluminum and silica, the assumption is quite reasonable. Bentonite is an extremely strong acid, its acid dissociation is very high, so much so that some samples of bentonite are electronegative over a wide pH range with no isoelectric point. In acid solutions the concentration of H ions is so high that combination with iron and aluminum is impossible, but occurs at pH 6.5, when the concentration of H ions is so much lower that the H ion easily dissociates from the colloid, permitting combination with the iron and aluminum.

As the H ions dissociate, when the suspension is made alkaline, the oxygen atoms are left with the lone pair of electrons, with which the H ions previously combined. The chlorides of iron and aluminum also dissociate, the OH groups of the alkali attaching themselves to the iron and aluminum through the lone pair of the oxygen atom. The dissociated colloid and the iron and aluminum hydroxides may be represented as follows:



Both the atoms of iron and aluminum are unsaturated, for they have only six valence electrons, whereas for stability two more are required, which is the exact number the oxygen atom has to offer; the following type of compound, therefore, is formed:



The combination or the bond is co-valent, throughout the compound, that is, the pair of electrons constituting the bond come from one and the same atom, the iron and aluminum obtaining six electrons from the (OH) group and a pair from the oxygen of the dissociated colloid. The octet is thus completed, the iron and aluminum achieving stability by increasing their valency from three to four. They, however, combine "head on," as it were, with the bentonite, are blocked by hydroxyl groups, and can no longer take part in base exchange reactions. The cause of the formation of the bond is the same as with the H ion, except that for iron and aluminum a group of eight is required for sta-

bility, a co-valency of four, but the H ion achieves stability with two shared electrons, the helium number, whereas iron and aluminum have to obtain the octet of the other inert gases.

Upon the combination of the iron and aluminum hydroxides with the lone pair of the oxygen, the number of OH ions at the surface of the colloid particle are increased by two for each atom of hydrogen dissociated. Since base exchange occurs with the displacement of this hydrogen by other atoms, an increase of base exchange capacity would be expected, but actually a decrease at pH 7.0 is obtained. This is because the isoelectric points of the iron and aluminum bentonites are at such a high pH, pH 6.5 for the ferric compound and pH 6.75 for the aluminum compound, whereas the bentonite itself is electronegative, dissociating cations at all ranges of pH. Bentonite, a strong acid, will have a considerable dissociation at pH 7.0; ferric bentonite, isoelectric pH 6.5, will come next; and aluminum bentonite, isoelectric pH 6.75, will have the least dissociation at pH 7.0, and hence the least base exchange capacity, which is not, however, a linear function of the pH. Apart from the fact that different compounds have totally different isoelectric pH values, there is no means as yet of determining what proportion of co-valent and electro-valent fractions exist at the isoelectric point of each compound; the dissociation is at a minimum, but the absolute amount may be different for each compound.

THE CLAY COMPLEX

Robinson and Holmes (13) studied the composition of a large number of American soils and examined the hypothesis that the primary product of weathering is an aluminum silicate $Al_2O_3 \cdot 2SiO_2 \cdot XH_2O$ of the kaolinite type, and a hydrated ferric silicate, non tronite $Fe_2O_3 \cdot 2SiO_2 \cdot XH_2O$; an excess of either sesquioxide or silica was found in most cases. G. W. Robinson (11), from the study of the composition of the clay fraction of Welsh soils, found that the data could, within limits, be represented on the assumption that the first product of weathering is a mixture of hydrated silicates of the general formula $R_2O_3 \cdot 2SiO_2 \cdot XH_2O$.

Considerable work in recent years has been done on the X-ray analysis of silicates by Bragg (2); on the clay fraction of the soil by Hendricks and Fry (4) and by Kelly, Dore, and Brown (6). The crystalline character of the clay fraction was demonstrated by comparison with the diffraction patterns of other known minerals, and some of the samples could be identified. Between these two conceptions, that of a colloid and the other a crystal, G. W. Robinson (12), in an interesting and critical discussion, finds us at a crossroad.

The distinction is not real, however, for the bonds in a colloid as in aluminum hydroxide, aluminum silicate, and from the evidence obtained from the iron and aluminum bentonite, in the natural bentonite as well, are co-valent bonds, arising out of one atom giving a lone pair of electrons necessary to form a single bond. To what extent the nature of the bonds can be determined by X-ray

analysis is very doubtful, for the silicates are very complex, and the interpretation of the data from this point of view is at this time only in its inception. In the case of the alkaline halides two types of structure occur, known as the rock salt and caesium chloride lattice. In the first type each sodium atom is surrounded symmetrically by six chlorine atoms and each chlorine by six sodium atoms; the second is similar except that the number is eight. This is taken as evidence that the force of attraction is electrostatic, with the sodium and chlorine existing as ions, so that there is no reason why a sodium atom should be more closely linked to one chlorine atom than to the other five. Such systems are, however, closely packed. If a system has an elaborate and open structure, occupying a large volume as in the soil colloid, it usually, as far as the evidence goes, points to co-valent bonds, but it is still crystalline in character. There is nothing incompatible with a crystalline character and dissociation in a polar medium. Sodium chloride is crystalline and dissociates in water; clay is also crystalline and dissociates in water. Although the degree of dissociation differs, its nature does not. An analogy can be drawn between strong electrolytes and weak electrolytes, such as sodium

TABLE 4

Base exchange capacity of ferric silicate with barium acetate solution at different pH values

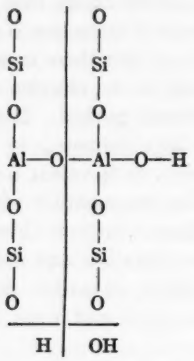
	pH OF BARIUM ACETATE		
	6.0	7.0	7.3
M.e. barium adsorbed per gram.....	0.627	0.981	1.141

chloride and acetic acid. An ionization of 100 per cent is almost certain for sodium chloride, an electro-valent salt, but acetic acid is co-valent with a small amount of electro-valent form. Replacing the H by sodium changes it into a strong electrolyte, and the molecular conductivities of sodium chloride and ammonium acetate are at moderate dilutions about 80 per cent of the values at infinite dilution, while that of acetic acid is only about 1 per cent.

For the interpretation of base exchange, X-ray analysis is not likely to prove very helpful, as base exchange is an ionic reaction that takes place between the colloid and a polar salt, and the determination of the isoelectric point and of the pH of the solution used for base exchange is much more important. The figures obtained for the base exchange capacity of a ferric silicate of $\frac{SiO_2}{Fe_2O_3}$ ratio of 3.20, on being leached with barium acetate solution at various pH values, show the increase in base exchange capacity with increasing pH of leaching solution (table 4).

Further, as the colloid ages, as previously described, by continual polymerization of the molecules, its empirical composition, that is, the ratio of silica to alumina, remains the same, but its base exchange capacity will be different

because the number of molecules taking part in the base exchange reaction are fewer. The number of molecules can be increased by hydrolysis, and Mattson (9) found that hydrolyzing the Sassafras colloid with NaOH, increased its base exchange capacity 300 per cent. This is actually a reversal of aging, the polymerized molecule breaking thus:



An equal ratio of $\frac{SiO_2}{Al_2O_3}$ is therefore no criterion of equal base exchange capacity, for in the soil polymerization and the reverse process of hydrolysis goes on, leading to profile development. The same colloid, however, whatever its degree of polymerization, will have the same isoelectric pH, for the surface bonds of the OH groups are the same whatever its age, and the isoelectric point is characterized by the pH when the colloid is either undissociated or dissociated amphoterically in an equal degree. The isoelectric point is therefore a much more fundamental point than the saturation capacity. The higher the base exchange capacity of any soil colloid as determined in the laboratory, the lower is its isoelectric pH and the higher the ratio of $\frac{\text{Acidoid}}{\text{Basoid}}$ but this is only true because aging is a relatively rapid process, and drying the colloid probably accelerates the change. If they were of different age it is quite conceivable that there would be no correlation between the isoelectric pH and the saturation capacity, because of the different degrees of polymerization.

SUMMARY

The increased activity of the H ion during the aging of aluminum hydroxide and silicate has been studied, together with the flocculation in barium acetate solution and diminished adsorption capacity on aging.

Both the hydroxide and silicate were synthesized by hydrolysis of the chloride, the oxygen of the hydroxyl group or the silicate group combining with the aluminum ion through the lone pair of electrons of the oxygen. This is

by definition a co-valent bond, but the molecules of the freshly prepared colloids are unsaturated, the aluminum having only six shared electrons but requiring eight for stability. The hydrogen, because of its small volume and consequent high mobility, dissociates in a water suspension, and the lone pair of the oxygen are transferred to the aluminum, increasing the number of shared electrons by two, the aluminum becoming quadrivalent and saturated. This leads to continuous polymerization until all the material is exhausted and gives an unsaturated molecule. The freshly prepared sol is stable in a solution of barium acetate, but the 3-weeks-old sol flocculates rapidly because of the increased size of the particles. It is therefore suggested that a particle of Ba-colloid may thus be actually smaller than that of a H-colloid, provided the barium is added to the freshly prepared colloid.

The bearing of this on current theories of base exchange and the structure of clay is briefly discussed. The strong adsorption of the H ion is attributed to the fact that it is the only cation that can form a co-valent bond with two shared electrons, whereas the other cations must be held by electrostatic attraction, as they require a group of eight for stability. Iron and aluminum when combined with bentonite are not afterwards removed by leaching with ammonium chloride, the combination with the lone pair of the oxygen taking place with the unsaturated hydroxides.

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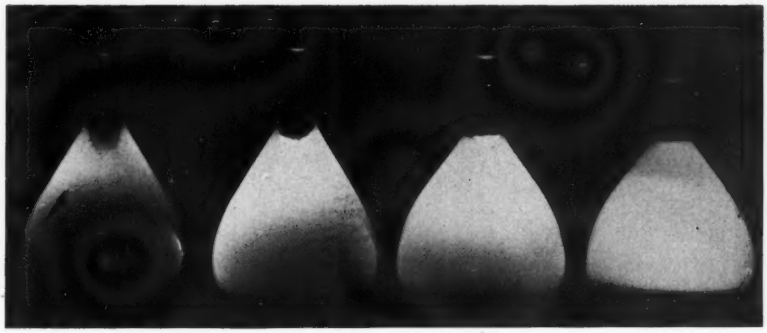
PLATE 1

EFFECT OF AGING ON THE STABILITY OF ALUMINUM SILICATE IN A SOLUTION OF BARIUM ACETATE

Age (reading from left to right): 0, 7, 14, and 21 days respectively